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par

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Effects of management and climate on the plant functional diversity related to ecosystem services of permanent grasslands in Europe

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Abstract
Permanent grasslands can provide a large diversity of ecosystem services. They are found in contrasted conditions in terms of management and climate in Europe. These conditions induce a wide variety of grassland types that differ in their vegetation and are expected to differ with respect to the provision of ecosystem services. Because ecosystem functioning in grassland has been shown to be linked to plant functional diversity criteria, we put forward that these functional diversity criteria can be used to evaluate the provision of ecosystem services. These criteria are influenced by the management and the climate. The overall objective of this PhD program is to assess the effects of management and climate on the plant functional diversity related to ecosystem services of permanent grasslands. The first step of the PhD program was to select 29 functional diversity criteria related to 8 ecosystem services (quantity of forage, forage quality, stability of the forage production, biodiversity conservation, pollination carbon sequestration, dryness resistance and nitrogen fertility). This selection was made using experts' interview and literature survey. The second step was to evaluate inaccuracies in the calculation of the 29 functional diversity criteria from functional trait databases and different sets of botanical surveys. The third step was to estimate the functional diversity criteria from management and climate data using “Random Forests” methodology. The results show that the climatic variables had generally more effect on the functional diversity than the management ones. Furthermore, for some climatic conditions, the management does not affect some functional diversity criteria. Among the 29 functional diversity criteria, only 8 have more than 40 % of their variance explained by the management and climate variables.
Keywords Functional diversity, climate, management, ecosystem services, permanent grasslands

Résumé
Les prairies permanentes peuvent fournir une grande diversité de services écosystémiques. Elles se trouvent dans des conditions contrastées en termes de gestion et de climat en Europe. Cette diversité de conditions induit une grande variété de types de prairies différant par leur végétation et leur fourniture en services écosystémiques. Certains travaux suggèrent que le fonctionnement des écosystèmes prairiaux peut être directement relié à des critères de diversité fonctionnelle végétale. Ces critères, qui pourraient être utilisés pour évaluer les services écosystémiques, sont influencés par la gestion et le climat. L'objectif de ce doctorat est d'évaluer les effets de la gestion et du climat sur la diversité fonctionnelle végétale liée à des services écosystémiques des prairies permanentes. La première étape a consisté à sélectionner 29 critères de diversité fonctionnelles liés à 8 services écosystémiques (quantité de fourrage, qualité du fourrage, stabilité de la production de fourrage, conservation de la biodiversité, pollinisation, la séquestration du carbone, résistance à la sécheresse et fertilité azotée). Cette sélection a été faite à partir d’interviews d’experts et d’une revue de la littérature. La deuxième étape a consisté à évaluer différentes inexactitudes dans le calcul des 29 critères de diversité fonctionnelle à partir de bases de traits fonctionnels et différents jeux de relevés botaniques. La troisième étape a consisté à évaluer les critères de diversité fonctionnelle à partir des données de gestion et de climat, en utilisant notamment la technique mathématique des forêts de Breiman. Les résultats montrent que les variables climatiques ont généralement plus d'effet sur la diversité fonctionnelle que celles de gestion. En outre, pour certaines conditions climatiques, la gestion n'affecte pas certains critères de diversité fonctionnelle. Parmi les 29 critères de diversité fonctionnelle, seulement 8 critères avaient plus de 40% de leur variance expliquée par la gestion et le climat.
Mots clés: diversité fonctionnelle, climat, gestion agricole, services écosystémiques prairies permanentes

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Résumé élargi en français de la thèse

A. Introduction

Les services écosystémiques sont les services rendus par les écosystèmes à l'humanité qu'ils soient ou non d'ordre économique. De très nombreuses recherches portent sur la fourniture des services écosystémiques. Celle-ci est cependant menacée par la dégradation des écosystèmes par l'humanité. Les activités humaines par ses émissions de gaz à effet de serre est responsable du changement climatique. Ce changement climatique a un fort impact sur le fonctionnement des écosystèmes. L'activité humaine est aussi responsable de la diminution de la biodiversité (extinction d'espèces ou homogénéisation des écosystèmes). La préservation de la fourniture des services écosystémiques est un enjeu majeur pour l'humanité. Différentes incitations ont été mises en place pour préserver et augmenter la fourniture de services écosystémiques. Pour les écosystèmes anthropisés, ces incitations sont sous la forme de subventions ou de labels.

Parmi les écosystèmes anthropisés, les prairies permanentes sont sans doute l'écosystème le plus important en termes de surface en Europe. De plus, les prairies permanentes apportent une grande diversité de services écosystémiques tels que la production de fourrage, la conservation de la biodiversité ou la séquestration de carbone. La fourniture en services écosystémiques varie entre les prairies permanentes. Il serait important de pouvoir évaluer les différences de provision de services écosystémiques.

La diversité fonctionnelle végétale pourrait être un bon indicateur de la fourniture des services écosystémiques des prairies permanentes. La diversité fonctionnelle peut être définie comme l'ensemble des valeurs de traits fonctionnels d'une communauté. Les traits fonctionnels sont des caractéristiques mesurables d'un individu qui sont reliées aux performances de cet individu.

De nombreuses études ont montré un lien entre diversité fonctionnelle et de nombreux services écosystémiques pour les prairies permanentes. Dans certaines études, la diversité fonctionnelle a déjà été utilisée comme indicateur des services écosystémiques. Cependant l'utilisation de la diversité fonctionnelle a certaines limites. La mesure de la diversité fonctionnelle est laborieuse et donc une évaluation rapide et avec une large échelle spatiale est impossible. De plus dans les prairies permanentes, la diversité fonctionnelle n'est pas directement gérée par les éleveurs seulement influencé indirectement à l'opposition des prairies semées (temporaire).

Il serait intéressant de pouvoir développer une méthode pour estimer la diversité fonctionnelle à partir d'autres facteurs comme la gestion et le climat. En effet ces deux facteurs ont une influence sur la diversité fonctionnelle végétale. De plus selon certaines études, les effets de la gestion sont différents selon les conditions climatiques. Les connaissances actuelles sur l'effet du climat et de la gestion sur la diversité fonctionnelle ne sont pas suffisantes pour permettre une estimation de la diversité fonctionnelle. En effet, les effets d'interactions entre le climat et la gestion n'ont été que peu étudiés. De plus, les variables de la diversité fonctionnelle liées aux services écosystémiques ne sont pas forcément les variables pour lesquelles les effets du climat et de la gestion sont les mieux connues. Peu d'études ont évalué la qualité de l'estimation de la diversité fonctionnelle à partir de la gestion et du climat.

L'objectif de la thèse était d'évaluer les effets de la gestion et du climat sur la diversité végétale fonctionnelle reliée aux services écosystémiques des prairies permanentes en Europe. Afin de réaliser cet objectif, la première étape fut de sélectionner une liste de critères de diversité fonctionnelle qui pourraient être utilisés pour évaluer les services écosystémiques des prairies permanentes.

Dans un second temps, nous avons testé les effets de la gestion et du climat sur les critères de diversité fonctionnelle sélectionnés.
Nous ne disposions pas d'estimations quantifiées du niveau de fourniture des services écosystémiques rendus par les prairies. Nous avons donc effectué la sélection des critères de diversité fonctionnelle en se basant sur la littérature et sur les expertises de chercheurs en écologie et en agronomie. Pour tester l'effet de la gestion et du climat, nous avons utilisé 3 jeux de données déjà obtenus provenant de différentes régions en France et en Suisse et contenant des informations sur le climat, la gestion et des relevés botaniques. Afin de calculer la diversité fonctionnelle, nous avons combiné les relevés botaniques et des bases de traits fonctionnelles.
Le manuscrit est séparé en trois chapitres : Le premier chapitre porte sur la méthode de sélection des critères de diversité fonctionnelle liés aux services écosystémiques des prairies permanentes. Le second chapitre porte sur différentes études des imprécisions dues à l'utilisation de bases de traits fonctionnels. Le dernier chapitre porte sur les effets du climat et de la gestion sur la diversité fonctionnelle.

B. Première partie : Sélection de critères de diversité fonctionnelle reliés aux services écosystémiques des prairies permanentes.

L'objectif de cette partie était de sélectionner des critères de diversité fonctionnelle reliés à des services écosystémiques. La première étape fut de choisir les services écosystémiques d'étude. Nous sommes partis d'une liste de services écosystémiques proposée pour les agro-ecosystèmes. Nous n'avons gardé que les services pertinents pour les prairies permanentes et qui seraient reliés avec la diversité fonctionnelle. Nous avons finalement sélectionné huit services écosystémiques (quantité et qualité de fourrage produits, stabilité des caractéristiques fourragères durant l'année, séquestration de carbone, fertilité azotée du sol, résistance à la sécheresse, pollinisation et conservation de la biodiversité).
Nous avons choisi des variables permettant d'évaluer chaque service. Nous avons ensuite identifié les processus écosystémiques impliqués dans la réalisation de ces services. Nous avons ensuite fait des entretiens avec des experts et une revue de la littérature pour trouver des critères de diversité fonctionnelle reliée à ces processus écosystémiques. Au final, vingt-neuf critères de diversité fonctionnelle ont été sélectionnés permettant d'évaluer huit services écosystémiques. Parmi ces vingt-neuf critères, dix était des pourcentages d'abondance relative de certains groupes de plantes (comme le pourcentage de légumineuses) et dix autres étaient calculés à l'aide de traits fonctionnels et d'indice de diversité fonctionnelle.

C. Deuxième partie : estimation des imprécisions dues à l'utilisation des bases de traits fonctionnels et de relevés botaniques.

L'objectif de cette partie était d'évaluer différentes erreurs liées à l'utilisation d'une base de traits fonctionnels et des relevés botaniques à la place de mesures de diversité fonctionnelle sur le terrain. Nous avons réalisé différentes études pour évaluer ces différentes erreurs dues à l'utilisation des bases de traits fonctionnels. Ces bases de traits regroupent de très nombreuses mesures de traits fonctionnels, mais tous les traits ne sont pas mesurés pour l'ensemble des espèces. Les bases de traits contiennent donc de nombreuses données manquantes. Des méthodes mathématiques peuvent être utilisées pour remplacer les données manquantes par des valeurs estimées: les méthodes d'imputation. Nous avons réalisé différentes simulations afin d'évaluer

l'importance des données manquantes dans le calcul de la diversité fonctionnelle. Nous avons d'abord évalué l'effet des données manquantes au niveau des espèces. Nous avons pour cela utilisé environ un millier d'espèces pour lesquelles aucune valeur ne manquait pour neuf traits fonctionnels. Nous avons ensuite supprimé aléatoirement certaines valeurs de traits et ainsi créé une série de bases de traits avec une proportion croissante de données manquantes. Différentes méthodes d'imputation ont ensuite été utilisées pour remplacer les données manquantes par des valeurs estimées: certaines très simples (remplacement par la moyenne ou la médiane des valeurs existantes), d'autres reposant sur des hypothèses écologiques (similarité fonctionnelle entre espèces ou corrélation entre les traits) et une méthode complexe basée sur une série de différentes imputations (multiples imputations en chaine). Nous avons ensuite comparé les valeurs de traits d'origine (avant la délétion des valeurs) et les valeurs de traits après les imputations afin d'évaluer la qualité du remplacement.
Parmi les méthodes d'imputation, les méthodes basées sur des hypothèses écologiques (relations entre les traits et proximité fonctionnelle entre les espèces) et la méthode d'imputation en chaine d'équation donnaient de meilleurs remplacements des données manquantes que les méthodes mathématiques simples. Le niveau d'erreur induit par les données manquantes dépendait de la distribution des valeurs de traits dans la base de traits. Plus la distribution des traits fonctionnels était normale plus la qualité du remplacement était bonne. Nous avons ensuite testé les effets des données manquantes et des méthodes d'imputation sur le calcul d'indices de diversité fonctionnelle. Pour cela nous avons utilisé un jeu de 700 relevés. Nous avons créé une base de traits avec les espèces présentes dans ces relevés. Afin d'avoir une base de traits sans données manquantes, nous avons fait une première imputation. Sur cette base de traits, nous avons effacé un pourcentage variable de valeurs de traits puis remplacé ces données manquantes avec différentes méthodes d'imputations. Différents indices de diversité fonctionnelles ont été calculés avec la base de traits sans données manquantes et les différents bases de traits avec des données manquantes imputées. Nous avons ensuite comparé ces deux types d'indices. En choisissant la bonne méthode d'imputation, la majorité des critères de diversité fonctionnelle peuvent être calculés avec 30% de données manquantes.
Dans les bases de traits fonctionnels, pas plus d'une valeur par trait est en général présente. Cependant, les traits fonctionnels peuvent varier fortement au sien d'une espèce. Cette variabilité intraspécifique ne peut donc pas être prise en compte lors de l'utilisation de la base de traits. Nous avons donc fait des simulations pour quantifier les effets de la variabilité intraspécifique sur le calcul de la diversité fonctionnelle. Pour cela nous avons utilisé 6 différents types de communautés (3 virtuelles et 3 jeux de communautés de prairies permanentes à des échelles spatiales différentes). Nous avons inséré virtuellement de la variabilité intraspécifique suivant 4 méthodes différentes (la valeur du trait dans la base est la moyenne du trait pour l'espèce, la valeur de trait n'est pas la moyenne du trait pour l'espèce, le trait de l'espèce varie de la même façon que le trait moyen de la communauté et le trait varie à l'opposé du trait moyen de la communauté). La variabilité a été insérée avec différentes amplitudes de 1% à 200%. Les critères de diversité calculés avec la variabilité intraspécifique ont été comparés avec les critères de diversité calculés sans variabilité intraspécifique. Sur l'ensemble des simulations certains critères étaient très affectés par la variabilité intraspécifique, d'autres moins. De ces résultats, nous avons pu établir trois règles pour évaluer l'importance de la variabilité intraspécifique sur le calcul de diversité fonctionnelle : (1) plus les valeurs du critère de diversité fonctionnelle sont variées plus le critère est robuste à la variabilité intraspécifique, (2) identifier le pattern de la variation intraspécifique (la manière dont le trait varie au sein

d'une espèce), si le trait au sein d'un espèce varie dans le même sens que le trait moyen de la communauté le long d'un gradient, le critère est moins robuste à la variabilité intraspécifique. (3) il faut évaluer l'amplitude de la variabilité intraspécifique du trait, plus le trait est variable moins le critère est robuste à la variabilité intraspécifique.
Une autre problématique avec l'utilisation combinée des bases de traits et de relevés botaniques est la présence d'espèces dans les relevés botaniques qui ne sont pas présents dans la base de traits (espèces non identifiées, espèces seulement présentes aux stades juvéniles dans les prairies comme les arbres, etc). Ces espèces non présentes dans les bases de traits peuvent avoir une abondance relativement importante et induire une erreur. Nous avons donc évalué quelle quantité de l'abondance dans un relevé peut être supprimée sans trop influencer le calcul de la diversité fonctionnelle. Pour cela, nous avons progressivement supprimé les espèces peu abondantes dans des relevés botaniques et comparé les indices de diversité fonctionnelle calculés sur ces relevés avec suppression et les relevés d'origines.
La dernière problématique que nous avons étudiée concernait les différences de protocoles. En fait dans les jeux de données que nous avons utilisés les protocoles de relevés botaniques différents en particulier en termes de surfaces de relevés. Nous avons effectué sur 16 prairies permanentes des relevés botaniques sur neuf surfaces différentes. Nous avons ensuite étudié les relations entre la diversité fonctionnelle et la surface de relevés botaniques afin de proposer des méthodes de correction et d'évaluer les erreurs dues aux différences de surfaces de relevés.
A partir de ces quatre études, nous avons proposé un niveau de précision de l'estimation de chacun des vingt-neuf critères de diversité fonctionnelle. Ce niveau de précision ne sera pas utilisé pour éliminer certains des critères de diversité fonctionnelle mais uniquement pour mieux comprendre les résultats de l'effet de la gestion et du climat sur la diversité fonctionnelle.

D. Troisième partie : Effets de la gestion et du climat sur la diversité végétale fonctionnelle des prairies permanents.

L'objectif de cette partie était de tester les effets de la gestion et du climat sur la diversité fonctionnelle végétale des prairies permanentes. Pour cela nous avons combiné 3 jeux de données existants. Le premier contenait les informations pour 140 prairies dans différentes régions de France (issu du travail sur la typologie nationale des prairies permanentes). Le second contenait 70 prairies du massif vosgien (issu du travail sur la typologie des prairies permanentes du massif vosgien). Le dernier contenait des informations pour 229 prairies dans deux régions des Alpes suisses. Les vingt-neuf critères de diversité fonctionnelle sélectionnés dans la première partie furent calculés à l'aide des bases de traits LEDA, TRY et Eflorasys. Dix-neuf variables de gestion et du climat ont été calculées sur l'ensemble des prairies. Six variables climatiques : l'altitude, la température moyenne entre avril et octobre, la température moyenne entre octobre et avril, la somme des précipitations pendant l'année, la somme des précipitations en été et une estimation de la production d'herbe potentielle. Huit variables décrivant l'utilisation de l'herbe : le type d'utilisation annuelle (seulement pâture, seulement fauche ou utilisation mixte), le type de la première utilisation (fauche ou pâturage), le nombre de fauches par an, le chargement annuel, le nombre d'utilisations, un index d'utilisation de l'herbe estimant le pourcentage du couvert végétal défolié au cours de l'année, un indice de perturbation et un indice relatif d'utilisation de l'herbe rapporté à la production potentielle d'herbe. Deux variables sur les dix neufs étaient des variables concernant la date de la première utilisation, la date en jour julien et le somme de

température à la date de la première utilisation. Et pour finir trois variables décrivant les apports en azote (minéral, organique et totaux).
Nous avons testé les corrélations entre ces variables. Les variables climatiques et celle de la période de première utilisation étaient fortement corrélées. Les variables d'utilisation étaient corrélées entre elles, ainsi que les variables de fertilisation. Le lien entre les variables de climat et celles d'utilisation-fertilisation était faible. Ces différents variables étaient très variables sur l'ensemble du jeu de données.
La première étape de notre analyse était d'identifier des tendances générales sur l'ensemble du jeu de données. La première étape fut de regarder les corrélations entre les 19 variables de gestion et du climat et les 29 critères de diversité fonctionnelles. Nous avons trouvé certaines tendances dans notre jeu de données. Par exemple, le nombre d'espèces végétales augmente avec l'altitude à l'inverse du trait agrégé de la teneur en azote des feuilles. Les mêmes tendances furent observées avec la date de première utilisation. Cependant ces résultats sont difficiles à interpréter en raison des fortes interrelations entre les variables climatiques et de gestion. Pour cela nous avons effectué une partition de variances pour voir l'importance des interrelations entre les variables de climat et de gestion. L'interrelation entre les quatre groupes de variables avait un fort pouvoir explicatif sur les critères de diversité fonctionnelle. Afin de pouvoir décorréler les effets des différents variables, nous avons utilisé des modèles d'équations structurelles. Nous avons testé différentes hypothèses de relations entre les différentes variables de climat, de gestion et de diversité fonctionnelle. Avec les modèles d'équations structurelles, nous avons pu séparer les effets de certains des effets du climat et de gestion. Cependant les modèles d'équations structurelles n'étaient pas suffisamment ajustées pour être totalement interprétés.
Nous avons aussi testé la présence d'effets conditionnels (l'effet d'une variable conditionnelle des valeurs d'une autre variable; interaction). Pour cela nous avons utilisé des analyses de la famille des arbres de régression. Nous avons d'abord utilisé une forêt de Breiman pour identifier les importantes variables de climat et de gestion. Le climat avait généralement plus d'importance dans l'estimation des critères de diversité fonctionnelle que la gestion. Parmi les variables de gestion, les variables d'intensité de gestion comme l'index d'utilisation de l'herbe avaient plus d'importance que les variables de types de gestion comme le type d'utilisation dans l'année. Nous avons ensuite construit des arbres de régression à partir des variables importantes identifiées par la forêt de Breiman. Ces arbres nous ont permis d'identifier des effets conditionnels. Par exemple, pour les prairies avec un hiver doux, nous n'avons pas trouvé d'effets de gestion sur certains critères de diversité fonctionnelle comme le nombre d'espèces végétales ou le trait agrégé de la teneur en azote des feuilles. L'estimation des critères de diversité fonctionnelle était meilleure lorsque les effets conditionnels étaient pris en compte. Certains des vingt-neuf critères de diversité fonctionnelle étaient très bien expliqués par les variables de gestion et de climat (nombre d'espèces végétales, trait agrégé de la teneur en azote des feuilles, de la date de floraison et la surface spécifique foliaire). D'autres critères étaient très mal expliqués par le climat et la gestion comme le trait agrégé de la hauteur végétative ou la teneur en matière sèche des feuilles.

E. Conclusions de la thèse

Nous avons proposé une liste de vingt neufs critères de diversité fonctionnelle qui peuvent évaluer huit services écosystémiques dans le chapitre1. Bien que certains liens entre diversité fonctionnelle et processus écosystémiques soient encore peu connus, nous avons pu établir cette liste en nous basant sur de

l'expertise et de la littérature. Ces informations pourraient être utilisées pour construire de véritables outils d'évaluation des services écosystémiques utilisant la diversité fonctionnelle. Nous avons aussi montré que certains de ces critères de diversité fonctionnelle pouvaient difficilement être calculés à partir de bases de traits fonctionnels. Si outils d'évaluation de services écosystémiques étaient disponibles, les résultats issus de notre travail sur les effets de la gestion et du climat sur la diversité fonctionnelle pourraient être utilisés pour estimer cette diversité fonctionnelle.

Nous avons en effet trouvé que certain de ces critères était fortement influencés par le climat et la gestion des prairies permanentes. Ces critères estimés pourraient être ensuite utilisés dans les outils d'évaluation des services écosystémiques (une évaluation en deux étapes).

Introduction

I. Ecosystems as a source of human well-being

A. Benefits that the ecosystems provide for mankind

Ecosystems provide various benefits for mankind and contribute to the human well-being. Human well-being can be considered as an unifying concept of the objective and subjective factors which constitute health and quality of life (Sarvimäki, 2006). **Ecosystem benefits** can be directly economic such as the sale of agricultural goods. Ecosystems benefits are also environmental benefits such as climate regulation or biodiversity conservation. These benefits have an economic impact but this impact is difficult to appraise (Fisher *et al.*, 2011). Some ecosystem benefits have no direct commercial value such as esthetic and ethical benefits.

Ecosystem services are the ecosystem's characteristics used to realize the ecosystem benefits. Ecosystem benefits are obtained by the interaction between human beings and ecosystem services (Boyd and Banzhal, 2007). A benefit may rely on several ecosystem services. The term “ecosystem services” was much popularized by the Millennium Ecosystem Assessment (MEA, 2003). The term “ecosystem services” is now used in a lot of publications especially in economy and in ecology (8 111 publications with the term ecosystem services in the web of science in May 2013) and a new journal named “Ecosystem services” was created recently (http://www.journals.elsevier.com/ecosystem-services/). The importance taken by the ecosystem services in research and in our society is due to the consequences of the strong degradations of ecosystems under human impact. These degradations induce a loss of ecosystem services and so of ecosystem benefits

B. Human impact on ecosystems

Human **activities have several impacts on ecosystem functioning** (Vitousek, 1997). These impacts are direct by replacing of native and complex ecosystems by simpler ecosystems (Foley *et al.*, 2005), or indirect by changing the environmental conditions.

In example, human activities induce **an increase of greenhouse gases (**CO_2, CH_4 ...) in the atmosphere with different consequences on ecosystems like climate change. Modification of the climate has a strong impact on the ecosystems (Vitousek, 1997).

Another strong impact of human activities on ecosystem is the **loss of biodiversity** (Sala *et al.*, 2000). This biodiversity loss comprises two different aspects. The first aspect is the **extinction of species at the global scale**. Under the human influence, the current extinction rate is 100 to 1000 times higher than in the general dynamic of biodiversity (Pimm *et al.*, 1995). The second aspect is the **simplification of ecosystems** (less species inside an ecosystem) and **biotic homogenization** (difference of species between ecosystems becoming less important) (McKinney and Lockwood, 1999). It was suggested that a more diverse ecosystem in term of species provides more ecosystem services (Grime, 1998; Hector *et al.*, 1999; Yachi and Loreau, 1999; Lehman and Tilman, 2000; Loreau, 2000, 2003; Hooper *et al.*, 2005; Diaz *et al.*, 2007a).

The preservation of the **delivery of ecosystem services** has to be **one major goal** for humanity during the current century. Measures are necessary to protect or/and to improve the provision of ecosystem services.

C. How to preserve or improve the provision of ecosystem services?

Different incentive schemes or regulations were proposed in order to maintain or improve the provision of ecosystem services (Kinzing *et al.*, 2011). Regulations are proposed to protect the "natural ecosystems". **Nature reserves** are one example of

these protections. Nature reserves are in concurrence with other human activities and so generally restrained to very small areas. This strategy of strict conservation cannot be the only way to protect or improve the global provision of ecosystem services (Gomez-Baggethun and Perez, 2011). **Ecosystems managed by humans also provide ecosystem services** (Costanza *et al.*, 1998). Managed ecosystems are agro-ecosystems, managed forests and aquatic ecosystems with fisheries or aquaculture. For these ecosystems, regulation goals have to favor management systems that induce ecosystem services.
The first way to improve the provision of ecosystem services is regulations in form of restriction. In several countries, **some restrictions in the management of these ecosystems are imposed by law**. One example is the limitation of fertilization in the area close to aquatic elements in EU (Directive91/676/CEE, 1991).
States also propose incentives in the form of subventions. **Subventions are generally linked to management specifications that are supposed to produce more ecosystem services** (late utilization of the grassland, presence of agro-ecological infrastructure).
The economic market also exerts incentives. **Certification is a way for the consumer to recognize "ecosystem services friendly" products.** A large number of labels exist with different specifications. Another option is to directly link the user of ecosystem services to the "manager" of the ecosystem. Users provide payment to the ecosystem managers in return of some obligations on their management (Pagiola, 2008).
These different incentives could be useful for the maintenance of ecosystem services. **To be efficient, the provision of ecosystem services has to be valued and quantified**. Economic valuation of ecosystem services relies on different aspects (Fisher *et al.*, 2011) (Sanchrico and Springborn, 2010). The second aspect is the quantification of the provision of ecosystem services and the understanding of its drivers (climate, management…). Quantification methods have to be proposed for all types of ecosystem.
Grassland is the most important managed ecosystem in term of surface (around 40 percent of terrestrial surface of the Earth) (Pedro Silva *et al.*, 2008) and is also important in term of quantity and diversity of ecosystem services it provides.

II. Grassland: an important but threatened provider of ecosystem services in Europe

A. Definition of grassland

Grasslands are "**lands covered by herbaceous plants** with less than 10 percent of tree and shrub cover" (UNESCO). Grasslands are generally managed for forage production. Several classifications of the grasslands can be made regarding the type of use (pasture and meadow) or the intensity of human influences (temporary grassland, semi-natural or natural grassland …). The most common distinction is made regarding the time during two renovations of the vegetal cover (reseeding). **Temporary grasslands are regularly reseeded** and generally inserted in a crop rotation. On the contrary, **permanent grasslands last for several years** without being reseeded and are not inserted in a crop rotation. However, the difference made between permanent grassland and temporary grassland may differ depending on the type of "users" (farmers, agronomists, ecologists and stakeholders) (Plantureux, 2012).
For the EU regulation, **permanent grasslands are not ploughed during at least 5 years**. This threshold is different between countries (Reheul *et al.*, 2007). In this PhD, we only focus on permanent grasslands using the EU definition.

B. State and dynamics of grassland in Europe

In the European Union (27 countries), permanent grassland represents 57 million ha (de Viellegher and Van Gils, 2010). **It corresponds to 13% of the total surface and 33% of the agricultural surface.** Percentage of permanent grassland is very different between countries. The permanent grassland represents more than half of the agricultural surface in some European countries (in and outside EU). For other countries, this percentage is very low (Finland 1%, Greece 7%, and Denmark 9%). The temporary grassland represents 9.7 million ha. From a geographical point of view (Figure.1), **the permanent grassland is present close to the ocean** (Ireland, Netherlands, North West of France), in these regions, grass productivity is high (de Viellegher and Van Gils, 2010). **Permanent grasslands are also found in mountain areas such as the Alpine regions**. In these regions, permanent grassland is the only sustainable type of agriculture because of the harsh climatic conditions and the difficult topography for mechanization (de Viellegher and Van Gils, 2010).

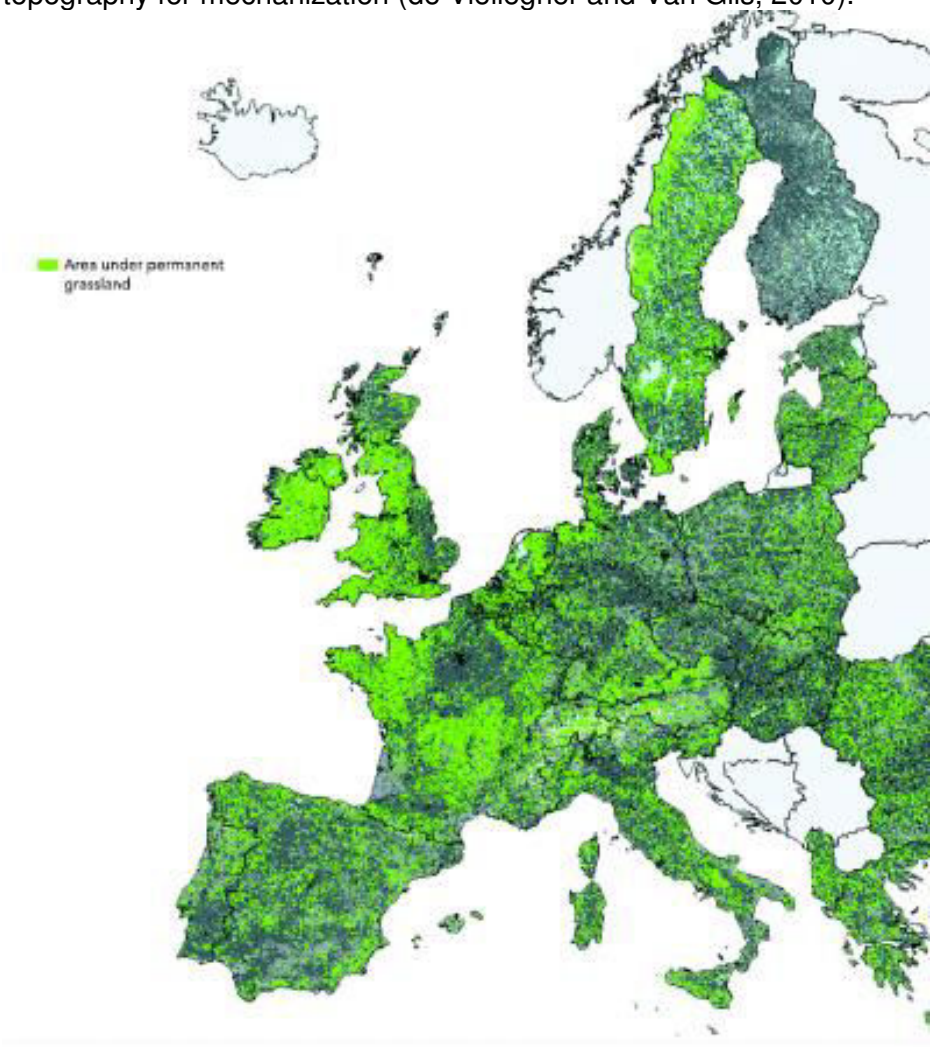

Figure1: Area under permanent grassland (light green) in most European countries (1995) (European Commission, 2008)

Between 1967 and 2007, **7 million ha of grassland disappeared** in the EU-6 (Belgium, France, Germany, Netherlands, Italy and Luxembourg). This decrease can be explained by the increasing use of **high yield forage crops like maize or temporary grasslands**. Moreover, the number of ruminants also decreased during this period (- 4.5 million cattle between 1983 and 2007), because of the milk quotas and the decrease of the consumption of beef meat (Peyraud *et al.*, 2012b).

In mountain areas, **unproductive grasslands are abandoned and naturally transformed into forests.** The transformation of agricultural land into urban and constructed areas also contributed to the decrease of grassland area.
However, this decrease has stopped since 2003. **The enforcement of new policies with some incentives to maintain grasslands is one reason to this stop.** But the increase of the price of the cereals may induce transformation of grasslands into crops. The decrease of grassland areas may have a consequence on the whole society by decreasing the provision of ecosystem services from grasslands.

C. Grassland ecosystem services

1. Forage services:

For farmers, forage production is the main service of the grassland area, as it provides a direct economic benefit. In order to harvest this forage production, the grassland can be directly grazed or cut to stock forage for the winter (and sometimes for the summer). Productivity of grassland is very heterogeneous depending on agricultural management, pedo-climatic conditions and botanical composition (Jeangros *et al.*, 1994). Permanent grasslands are generally less productive than other forage crops. However, some permanent grasslands reach the productivity of temporary grasslands (Delaby *et al.*, 1996; Launay *et al.*, 2011). **The quantity of forage production is not the only factor describing forage production.** The timing of biomass production is also important, especially at the farm scale. In the case where all the plots have to be harvested at the same time, the workload would be high and forage would be lost. Possibility of grazing during the summer is also important. Absence of grass production during dry periods in summer would oblige farmers to use stored forage. **Forage quality is also an important aspect of forage production.** Forage quality is generally described by the nutrient content (energy, protein, …) and the digestibility of the forage (Baumont *et al.*, 2012). The quality of animal products (cheese, meat…) can be linked to the presence of some plant species in the grassland (Farruggia *et al.*, 2008). Some cheeses can only be produced with some type of forage to maintain the quality and the typicality of the product like the "Comté" (Décret n°2007-822, 2007) .

2. Environmental services:

Water quality depends of the load of pesticides and nutrients entering the ground and surface waters by leaching or runoff. For grasslands, the utilization of pesticides is very low in comparison to other crops (Raison *et al.*, 2008). Grasslands also have a limiting effect on phosphorus leaching (Le Gall *et al.*, 2009) and the leaching of nitrates is very low on extensively managed grasslands (Vertes *et al.*, 2010; Peyraud *et al.*, 2012a), and only occurs for high rates of nitrogen fertilization (more than 150 N.ha-1.year-1).
Permanent grasslands are very important in terms of carbon sequestration. They can be a sink for carbon, and a sequestration 0.5 to 0.7 t of C ha^{-1} $year^{-1}$ has been reported (Peeters and Hopkins, 2010).
The presence of vegetal cover during the whole year induces low erosion. The loss of soil by erosion is much less under a grassland that under a crop culture (0.3 versus 3.6 t $ha^{-1}year^{-1}$) (Cerdan *et al.*, 2010).
The increase of organic matter in the soil below grassland (Arrouays *et al.*, 2002) is one of the aspects of the contribution of grassland to soil fertility. The presence of nitrogen-fixing species (legumes) in grassland also has a positive effect on soil fertility. Nutrient recycling differs between grasslands depending on the differences in vegetation (Amiaud and Carrère, 2012).

3. Biodiversity conservation and tourism/esthetic value:

Grasslands have a higher biodiversity in comparison to forage crops. First, the plant biodiversity of grassland is high, especially for the old permanent grasslands. The intercommunity diversity (β diversity) is also very high: more than thousand species were found in grassland botanical surveys conducted in West Europe (Plantureux and Amiaud 2010). **Grassland can also contain endangered plant species**. The grassland procures a habitat for a wide range of animal taxa. Some of them are only found in grassland communities (Isselstein *et al.*, 2005). A lot of birds and butterflies species depend on grassland habitats (Sanderson *et al.*, 2008; Bruckmann *et al.*, 2010).
Grasslands create typical landscapes. They are considered the society as more natural than crops and are generally preferred in terms of esthetic (Bugalho and Abreu, 2008). In some regions, the presence of grasslands is a key point for the tourism (Mountain area, "bocage", and wetland). Finally tourism is often associated with local typical products produced on grassland (cheese and meat).
G**rasslands thus provide a large set of ecosystem services**. This multi-provision of ecosystem services is called multifunctionality (Le Goffe, 2001). The value of the ecosystem services provided by a grassland was estimated at 600 € ha^{-1}(Chevassus-au-Louis *et al.*, 2009). However a large diversity of types of permanent grasslands exists (from the sea level to more than 2000 meters). **Some of the previous services only depend on the presence of a vegetal cover while other rely on the vegetation characteristic and so would be very different between the different types of grasslands.** To improve the assessment of the ecosystem services and propose new incentive schemes, the evaluation must therefore take this diversity of types of grasslands into account.
The numerous relationships found between the diversity of the vegetation (functional or taxonomical) and some ecosystem functions motivated some authors to propose the utilization of this diversity to assess ecosystem services.
The understanding and the evaluation of these different services would be a key issue to propose new subventions and so to maintain grassland. A FP7 project (MULTISWARD project) was financed by the EU to improve the knowledge on the effects of plant diversity on grassland multifunctionality and propose evaluation tools.

D. The Multisward project

The aim of the **FP7 MULTISWARD research project** (www.multisward.eu) is to support developments and innovations in grassland use and management in different European farming systems (including low-input and organic), pedo-climatic and socio-economic conditions.
MULTISWARD goals were to (i) enhance the role of grasslands at farm and landscape levels to produce environmental goods and to limit the erosion of biodiversity and (ii) to optimize economical, agronomical and nutritional advantages for the development of innovative and sustainable ruminant production systems. One objective of MULTISWARD is to assess ways of combining high production efficiency with optimal provision of regulating and supporting services from grasslands at farm to regional levels.
The first step to this evaluation is to quantify the provision of ecosystem services. The next part of the Introduction presents different possibilities to quantify ecosystem services. This PhD was funded by the project.

III. Quantification of the ecosystem services

A. Direct quantification: ecosystem services and process measurements

The measurement of ecosystem services rely either on **direct measurements of services or on estimations of the ecosystem processes and functions related to these services.** The measurement of the processes and functions are useful to understand the effect of global changes on ecosystem functioning and then on ecosystem services. Examples of direct measurements of the production of goods, of services supported by the biogeochemical cycles and of biodiversity conservation are presented below.

1. Measurements of the production of goods

Production of goods may be directly **quantified or estimated by the quantity of goods harvested.** However, the harvest quantity is not always representative of the quantity of goods presents. For grasslands, the real quantity of forage produced can be underestimated in case of late harvest (senescent parts of plants not harvested). The estimation of the biomass production of grazed plots is also laborious.
Another difficulty is to compare the **production of goods between different types of ecosystems**, even for the good. It is hard to compare the production of a monoculture and a polyculture (intercropping and agroforestery for example). The use of only the harvest quantity of one species always under-evaluates the production of the polyculture. Indicators are proposed to assess the production of mix cultures like the land equivalent ratio (Mead and Willey, 1980). For grassland, if one species is harvested for other purposes (spices, medicinal herbs) this problem will occur between the evaluations of the forage production and the harvest of these species. Another way is to calculate the economic value of all the goods provided (Correia *et al.*, 2010).
The ecosystem services of goods production do not only concern the quantity of goods but also the quality of the goods produced. **The quality may be evaluated by numerous indicators**: some very objective (quantity of energy, content of some molecule, economics value…) and more subjective (taste, color, typicality …) (Warmke *et al.*, 1996; Kubícková and Grosch, 1998).

2. Measurements of the ecosystem services linked to biogeochemical cycles

Several ecosystem services rely on the biogeochemical cycles. Some of these ecosystem services can be directly measured, like carbon sequestration (increase in soil carbon) (Conant *et al.*, 2001) or erosion (quantity of sediments in runoff water). Some other services can only be quantified by the measurement of the processes related to these services. The measurement of the processes/functions between atmosphere and the plant-soil complex are generally studied by the gaseous exchanges between these two pools (Goulden *et al.*, 1996; Novick *et al.*, 2004).

3. Measuring the ecosystem services linked to biodiversity conservation

Biodiversity cannot be measured as a whole. Indeed, **biodiversity is the concept describing the diversity of all forms of life**. It is always measured for only parts of its components and is often described by the number of plant species..
Measurements cannot be realized on all the different communities present in an ecosystem. Subsampling is generally used to quickly assess biodiversity, which induces a reduction of the quality of the information (Lagache *et al.*, 2012). Biodiversity is sometimes assessed by the abundance or the dynamics of one or several species.
Because of the large differences in term of protocol and the intensity of work and materials necessary to measure ecosystem services, it would be very difficult to have a measurement of a set of ecosystem services for a large number of grasslands. Therefore, **an indirect measurement/evaluation of ecosystem services is usually**

necessary. Several authors propose that biodiversity can be used as an indicator of ecosystem services (see next paragraph).

B. Plant biodiversity as an indicator of ecosystem functioning and ecosystem services

In ecology, a major assumption is that biodiversity supports of ecosystem services and positively affects the ecosystem functioning (Solbrig, 1991; Naeem *et al.*, 1994).

1. Theoretical hypothesis beyond the positive effect of biodiversity on ecosystem functioning

Different mechanisms can explain this positive effect of biodiversity. The first mechanism is the complementary effect. **With a high biodiversity, more ecological niches are present in the community.** More ecological niches may induce a complementarity in term of resource uptake (Hooper, 1998). In more diverse communities, the number of different root systems is high and so the competition for the soil nutrients would be less important. Plant biomass and other processes may be higher. Secondly, with a higher biodiversity, **the probability to have a species (or individuals) with a higher performance will be more important** (Hector *et al.*, 1999). A higher biodiversity may also increase the probability of positive effects between two species.
The last hypothesis is the insurance hypothesis (Yachi and Loreau, 1999; Mouquet *et al.*, 2002; Loreau, 2003). Highly diverse communities may be more able to resist change. With more diversity, it is more likely to find a species adapting to the change. The diversity would thus allow maintaining the functioning of ecosystem in changed environnement condition.

2. Links between the number of species and the ecosystem functions

The first studies used the **number of species as descriptor of biodiversity** (Hector *et al.*, 1999; Roscher *et al.*, 2005).
In the majority of the experimental studies, the **number of species has a positive effect on the productivity of the ecosystem**, on the resistance to invasion and globally on ecosystem functions. Positive effect is due for one part to the sampling effect and for another part to the complementarily effect (Hector *et al.*, 1999). However *in natura*, the **productivity of ecosystem and the number of species is negatively linked** (Mittelbach *et al.*, 2001). The same paradox was also found for the resistance to invasive species: the number of invasive species is higher in very diverse ecosystems (Naeem *et al.*, 2000).
Regarding these opposite results, a global synthesis was performed (Hooper *et al.*, 2005). **The missing gap between these two types of observations was the effect of the environment on ecosystem functioning and on biodiversity.** Indeed, the best environmental conditions for a high biomass production (fertility soil, disturbance regime) are also the worst environmental conditions for high specific richness. So the **general trend between biodiversity and production is negative**. However, in the same conditions, **biodiversity may have a positive effect on the ecosystem functioning** (Figure.3).

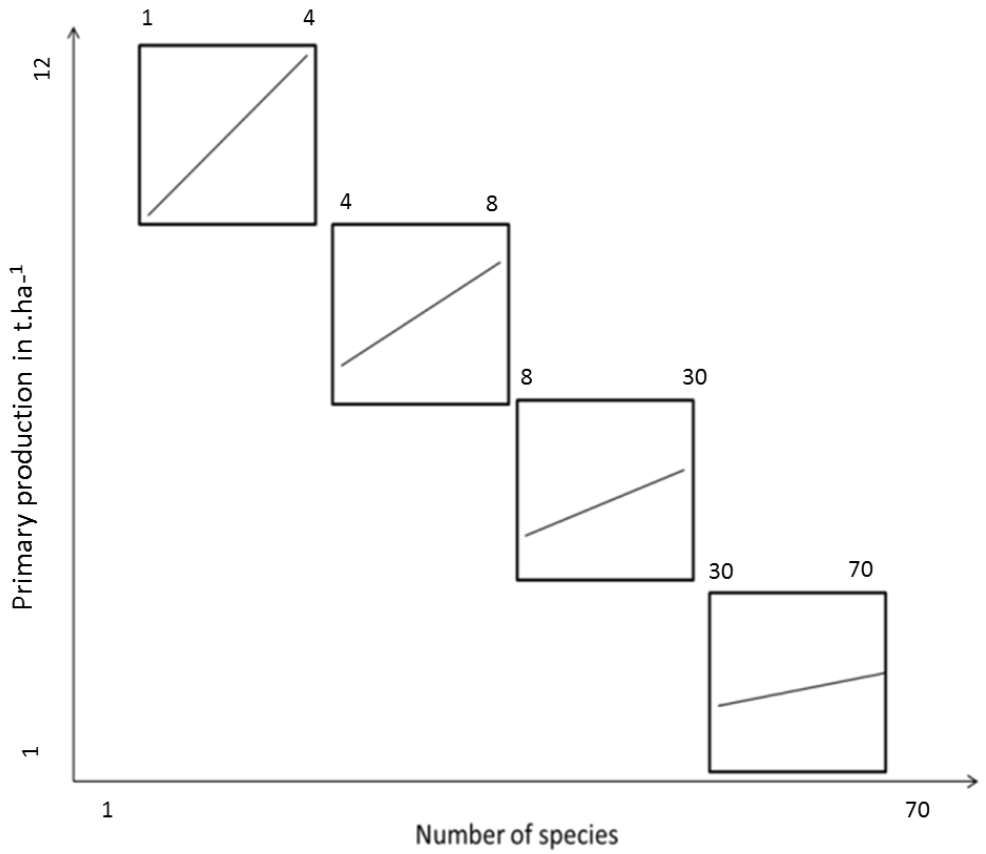

Figure.2: Relation between the number of species and the ecosystem functions. The black square represents community with the same environment. The values are only here as example.

A major limitation of the experiments on the links between the ecosystem function and biodiversity is **the use of the number of species** (Hurlbert, 1971). It supposed that the difference between two species in term of functioning is always the same between all the species. This major assumption may be one of the reasons of the low power of explanation of the relationships between biodiversity and ecosystem functioning. Another limitation is that the number of species does not take account the species abundance.

3. Functional approach of the biodiversity: functional groups diversity

Rather than regrouping plants individuals regarding their taxonomy, functional regrouping can be also made. **A functional group gathers species/individuals with the same function** (Lavorel *et al.*, 1997).
Species can be regrouped regarding **their effects on the ecosystem functioning** (effect group) or species with the **same responses to an environmental factor** (responses group). One interest of functional groups approach is to be more general and mechanistic than a taxonomic approach (Diaz and Cabido, 2001).
Complementarity effect is assessed by the number of functional groups and the "sampling effect" is assessed by the functional identify (presence of some functional groups). The "functional groups" approaches were used to study several ecosystem processes (Diaz and Cabido, 2001). Functional groups are also very often used for the design of experimental protocols.
However, the **functional group approach is also limited by the number of groups** (generally low) and by the method of regrouping species (Wright *et al.*, 2006). An *a priori* classification is generally used. The classification is made regarding the existing knowledge. So, this classification may be subjective.

Another way to use a multivariate classification method on functional traits to create functional groups (Petchey and Gaston, 2006). This is an *a posteriori* classification based on the measurement of the functional traits of the different species. A functional trait is any morphological physiological features measurable at individuals' scale which impacts the fitness indirectly via its effects on growth, reproduction and survival (Violle *et al.*, 2007).
However, to transform continuous information (functional trait) into a discrete measure would be a loss of information. A continuous approach is therefore more representative of the reality.

4. A continuous approach of functional diversity

For the continuous approach, functional diversity can be defined as the set of values of a functional trait taken by the individuals/species within a community. Different components of continuous functional diversity are described in the literature.

a. The different continuous component of functional diversity

Functional diversity can also be evaluated using together several traits (Mason and de Bello, 2013). Functional diversity can be subdivided **in 4 independent components**: functional identity, functional richness (or amplitude), functional evenness (or regularity) and functional divergence. Functional diversity indexes are the calculation methods allowing the quantification of one functional diversity component. The indexes are computed using the functional traits values by the species/individuals and their abundances.
Functional identity (FI) corresponds to the mean value of the functional trait of the individuals in a community (Figure.3a). This value is a way to sum up all the trait values creating an average individual of the community. Community weighted mean value is an index able to evaluate the functional identity of a community (Grime, 1998; Lavorel *et al.*, 2011).
Functional richness or amplitude (FR) corresponds to the range of the value taken by the functional trait (Figure 3c). When the trait is continuous, functional richness is the amplitude of functional trait (maximum minus minimum value). When the trait is discrete, functional richness is the number of modalities of the functional trait (i.e. number of modalities). Functional richness can also be evaluated with several functional traits. In this case, functional richness corresponds to functional space occupied by the community.
Several functional richness indexes exist. Some are based on the amplitude of functional traits: functional range (Mason *et al.*, 2005), convex volume (Cornwell *et al.*, 2006) or FRis index (Schleuter *et al.*, 2010). Others indexes are based on the functional dissimilarities between species: FAD (Walker *et al.*, 1999), FD (Petchey and Gaston, 2002) modified by Mouchet el al.(2008) and the number of groups created using dissimilarities (Petchey and Gaston, 2006).
Functional evenness or regularity (FE) corresponds to the distribution of the abundance inside the functional space (Mason *et al.*, 2005; Mouillot *et al.*, 2005; Villeger *et al.*, 2008) (Figure.3b). If the abundance is uniformly distributed between the different values of the functional trait, the evenness will be high. At the opposite, if the abundance is only distributed around one value, the functional evenness would be low. The evenness can be measured by the Shannon index when the function trait is discrete. For continuous trait, the FROm index (Mouillot *et al.*, 2005) can be used for one trait and the FEve index for multi trait approach (Villeger *et al.*, 2008).
Functional divergence (FV) corresponds to the repartition of the abundance regarding the functional identity in the functional space (Mason *et al.*, 2005; Villeger *et al.*, 2008; Mouchet *et al.*, 2010) (Figure.3d). If the abundance is distributed on the extreme values of the functional space, functional divergence would be high. In contrast, if most of the abundance is concentrated around the mean value, the functional divergence would be low.

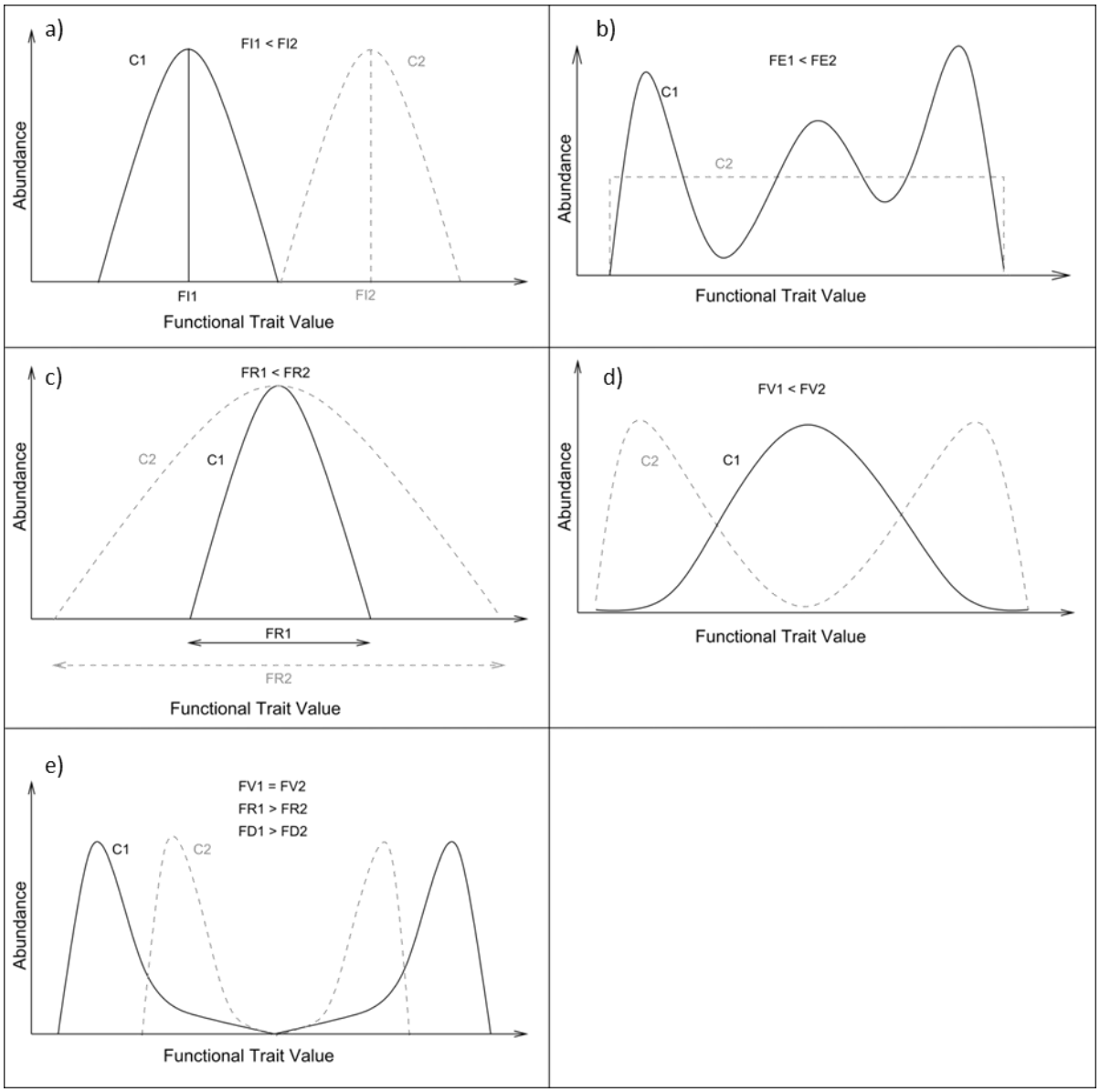

Figure 3: Schematic illustration of the different components of functional diversity. The x-axis represents the value of one functional trait; the y-axis represents the abundance of the species inside the community at the different values of the functional trait. In each aspect, two example communities (C1 and C2) with opposite functional diversity situation are represented. a) Functional identity (FI), b) Functional evenness (FE), c) Functional richness(FR), d) Functional divergence(FV), e) Functional dispersion(FD)

Functional divergence is related to the rules of repartition of abundance inside the communities and the number of functional strategy. When only one functional strategy is dominant, the divergence is low. When at least two strategies are dominant, the divergence is high. For the divergence of one trait, two indexes can be used, the mason index (Mason *et al.*, 2003) and the FDis index (Schleuter *et al.*, 2010). For the calculation of the divergence with several traits, only the FDiv index is available (Villeger *et al.*, 2008).

Functional dispersion (FD) is a new concept (Laliberté and Legendre, 2010). This is a combination of functional richness and functional divergence. Rao index and the FDisp index can be used to calculated functional dispersion (Figure.3e).

b. The links between functional diversity and ecosystem services

The community weighted mean value transcribes the **biomass ratio hypothesis** (Grime, 1998): The more a species is abundant, the more its effects on the ecosystem

is important. The presence of the species is not the only key parameter but its abundance must be taken into account. The biomass ratio hypothesis is a little bit different to the “sampling effect” where only the presence of the species is important (not the abundance).
For the aspect of complementarity, functional richness, evenness, divergence and dispersion could be good indicator. Indeed, these indicators take into account the distribution of the abundance in the functional space.
This continuous approach strongly improves the relation between the biodiversity and ecosystem function (Mason *et al.*, 2003; de Bello *et al.*, 2010).
Functional diversity with a continuous approach seems more related to the functioning of ecosystem than species richness and functional groups (Mason *et al.*, 2005; Mouillot *et al.*, 2005; Mouillot *et al.*, 2011). A review of the different relationships between functional diversity and ecosystem functions was made by de Bello et al (2010). In this review, most studies were made on biogeochemical process such as decomposition, fodder productivity, evapo-transpiration (Figure.4).

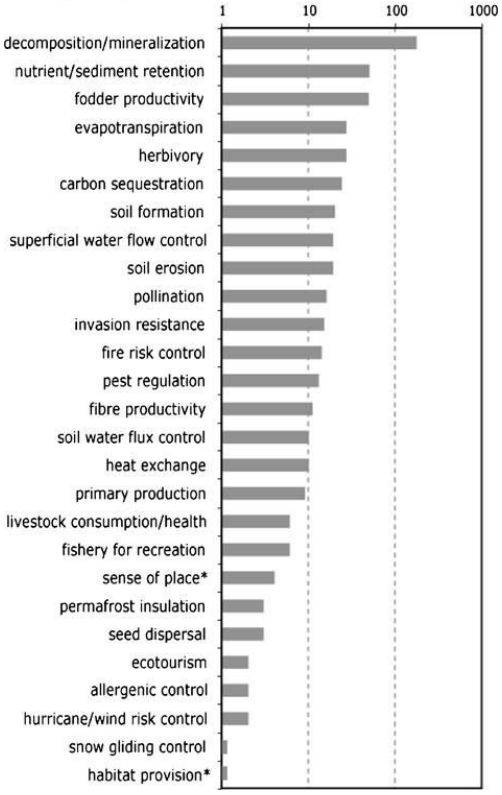

Figure.4: Distribution of trait–service relationship information by ecosystem processes assessed. Quantitative axis refers to number of studies that show relationships between functional diversity and ecosystem processes (log scale) (de Bello *et al.*, 2010).

Functional diversity of the **functional traits related to vegetative growth** such as specific leaf area, leaf dry matter content would be related to the biogeochemical ecosystem services such as the forage production, carbon sequestration, soil fertility (Garnier *et al.*, 2006; Mokany *et al.*, 2008; Klumpp and Soussana, 2009; Lavorel *et al.*, 2011). The biodiversity conservation services (animals and plant biodiversity, pollination) would be related to the functional diversity of the **traits related to reproduction** (such as onset of flowering and type of flowering) and to the **taxonomic diversity** such as the number of plant species or the percentage of some botanical families (Lavorel *et al.*, 2011).
However, the **links between functional diversity and the ecosystem process/function are not adequately understood.** In addition the variables of functional diversity (functional trait and indexes) used are not the **same between studies.** For the above biomass productivity, some studies show that functional identity is linked to the biomass (Lavorel *et al.*, 2011; Lavorel and Grigulis, 2012), wheras other

studies show an effect of functional divergence (Mokany *et al.*, 2008; Klumpp and Soussana, 2009; Mouillot *et al.*, 2011) or an effect of functional regularity (Mouillot *et al.*, 2005; Mokany *et al.*, 2008). Also, the functional traits are different between the studies. In some studies, several functional traits were used (Mouillot *et al.*, 2005; Mokany *et al.*, 2008; Mouillot *et al.*, 2011) or only one trait (Klumpp and Soussana, 2009; Lavorel *et al.*, 2011; Lavorel and Grigulis, 2012). The functional traits used are sometimes the specific leaf area (Klumpp and Soussana, 2009) or the vegetative height or the leaf nitrogen content (Lavorel and Grigulis, 2012). **No general consensus can be found to explain the link between functional diversity and biomass productivity**.
Based on the literature, we proposed a conceptual model with hypothesis on the link between the ecosystem services and functional diversity (Figure 5).

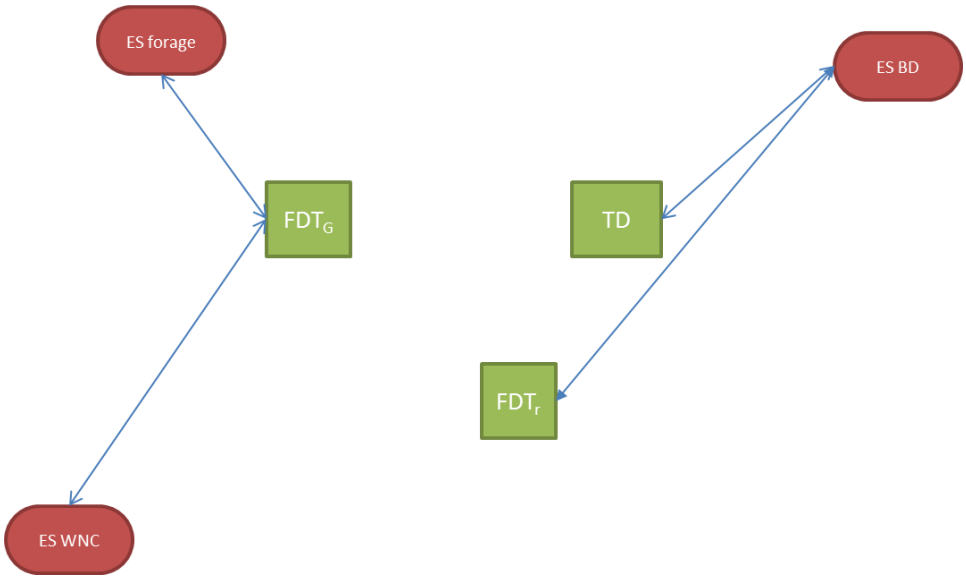

Figure 5: Conceptual schema based on hypotheses on the relationships between ecosystem services and functional diversity. FDT_G: Functional diversity of trait linked to growth. FDT_R: Functional diversity of traits linked to reproduction. TD: Taxonomical diversity. ESFORAGE: Ecosystem services related to forage production. ESWNC: Ecosystem service related to nitrogen, carbon and water cycle. ESBD: Ecosystem services related to biodiversity conservation. The blue arrow represented the links between ecosystem services and functional diversity

Another **problem in the theory** linking biodiversity and ecosystem services is the status of functional diversity on these relations. Does functional diversity cause the ecosystem functions? For example, the legumes by the nitrogen fixation have an effect on nitrogen cycle on the soil. Does the functional diversity results of the ecosystem functioning? For example, the leaf dry matter content is related to soil fertility (Ordoñez *et al.*, 2009) and so to the nutrient cycles. The link between functional diversity and the ecosystem functions could also be result of the effect of other factors. For example, rainfall and temperature have a positive effect on specific leaf area and also biogeochemical process (Wright *et al.*, 2004).
In agronomy research, the vegetation descriptors (not necessary functional diversity ones) are already used to evaluate the provision of some services of grassland. The next part of the introduction presents the existing tools using vegetation and their limitations.

C. Utilization of vegetation criteria in evaluating tool of ecosystem services

One example of the evaluation tools using vegetation criteria of ecosystem services is the grassland typology. The aim of a **typology is to classify grassland in types**. Within a type, the provision of ecosystem services is considered as equivalent. The evaluated ecosystem services differ between the different existing typologies. Some

typologies evaluate only forage services. Others also evaluated environmental services such as biodiversity or carbon sequestration; see Michaud *et al.* (2012a) for a review. The typologies are generally created using the same method: a set of grassland is used with ecosystem services and variables of the vegetation such as functional or taxonomical diversity. The vegetation variables can be derived from the botanical composition (presence and abundance of key species...) or/and functional characteristic of the vegetation. Using this data, groups of plots, with the same provision of ecosystem, are created using vegetation variables. The vegetation could be an input of the typologies and use for the evaluation. Management, soil and climate are often inputs to the typologies.

Typology can also be made at species level. Duru *et al.* (2012a) created a species typology based on the combination of 6 plant traits. This approach is close to the functional group's approach. This functional typology is constructed for 36 grass species. The presence and the dominance of the different functional types of grass are used as a tool to evaluate some services (forage production, quality, and stability).

These tools can be used directly from a botanical survey and associated with software such as e-Flora-sys (http://eflorasys.inpl-nancy.fr) to propose an agro-ecological evaluation from botanical surveys (Plantureux and Amiaud 2010). These typologies have some limitations.

The grass typology proposed by Duru *et al.* (2012a) only relied on grass species. The forbs and the legumes species have also an important role on the functioning of the vegetation. This typology must be completed with an evaluation of the percentage of the legumes and forbs (Baumont *et al.*, 2012). Another limitation is the relatively low number of species (36) described.

For the other typologies, Some ecosystem services like carbon sequestration, animal biodiversity conservation, esthetic value, animal product quality are present only on one local typology :the typology AOC massif central (Hulin *et al.*, 2011). The services like soil fertility, water regulation, drought resistance, erosion limitation are never assessed with these tools. **The scale of typologies is generally local and sometimes national.** The existing tools are quite adapted for theirs utilization at local scale (management recommendation).Typologies are limited to the evaluation of existing grasslands but they are not able to deal with a change of management or climate. Typologies are also generally empirically made and the mechanistic linking the studying services and the input factors are not generally explained (Michaud *et al.*, 2012a). **Tools using functional diversity and implemented with the more recent knowledge would be interesting**. Most of these typologies need botanical surveys as inputs that are time consuming.

To find a **simplest way to predict or at least estimate the vegetation** (functional diversity) criteria at the origin of ecosystem services would improve the evaluation of ecosystem services. The management and the climate have a strong influence on the vegetation. **The links between management and ecosystem services through functional diversity were already tested in some studies** (Klumpp and Soussana, 2009; Lavorel and Grigulis, 2012; Lienin and Kleyer, 2012). A framework was proposed to add the effect of different drivers on functional traits (responses traits) and the effect of traits on ecosystem functioning to evaluate ecosystem services (Lavorel *et al.*, 2013). To understand and estimate the effects of management and climate on functional diversity would improve the evaluation of ecosystem services.

IV. Effect of management on functional diversity of grassland

The effects of the management and the climate on plant diversity are already assessed in many studies especially for the specific richness (Gaujour *et al.*, 2012) In the following paragraph, the aspect on species diversity is not developed.

A. Selection of functional trait at individual scale by management and climate

The effects of management and climate on the functional diversity were first approached by the study of the **functional trait at individuals/species scale. In wet conditions**, species with a high specific leaf area are more frequent (Wright *et al.*, 2004; Wright *et al.*, 2005b). Soil fertility influences the **leaf economic spectrum functional trait**s like the specific leaf area, leaf dry matter content and leaf nitrogen content. Soils with high nitrogen content select plant species with high specific leaf area (SLA), high leaf nitrogen content and low leaf dry matter content (Ordoñez *et al.*, 2009). Grazing selects species with small stature and high SLA (Diaz *et al.*, 2001; Diaz *et al.*, 2007b). These different factors influence the functional strategy. A functional strategy is a set of functional traits. **Two main types of functional strategies are described in the literature**. Grime (1974) proposed functional strategies based on the concept of disturbance and constraint. **Disturbance is a factor that induces a loss of vegetal biomass**. **An environment factor is a stress if it limits the growth of the plants**. Ruderal species are adapted to high disturbance and low constraint conditions. Stress tolerant, at the opposite, are adapted to high constraint and low disturbance. Competitor species eliminate the other species in conditions where the disturbance and the constraints are low. These different strategies have different value of functional traits (Westoby, 1998; Diaz *et al.*, 2004). For example, the competitor species have a higher vegetative height than the ruderals. Another functional strategy relies on the nutriments conservation /acquisition trade-off. This trade-off is often defined as the leaf economics spectrum (Wright *et al.*, 2004). Leaf economics spectrum is influenced by the disturbance (for example high SLA occurs with high level of disturbance) and by constraints (SLA and LNC is low in high constraints like dryness or limitation in nitrogen).
Functional traits of species/ individuals present in the community result of the selective effect of different ecological filters (Keddy, 1992; McGill *et al.*, 2006).

B. Effect of management and climate on functional diversity

At community level, the effects of management and climate on functional identity (community mean value) are generally identical with the effects found at species level (Laliberté *et al.*, 2010; Lavorel *et al.*, 2011; Michaud *et al.*, 2012b). For the functional richness (the difference between the extreme value of the functional trait), the grazing could create a higher function richness of SLA but only under some condition of climate (de Bello *et al.*, 2005a, 2006). For the divergence (distribution of the abundance inside the functional space), higher divergence, for the trait linked to the leaves economics spectrum, are found for intermediate level of fertility, climate and disturbance (Duru *et al.*, 2009; Bernard-Verdier *et al.*, 2012; Duru *et al.*, 2012b).

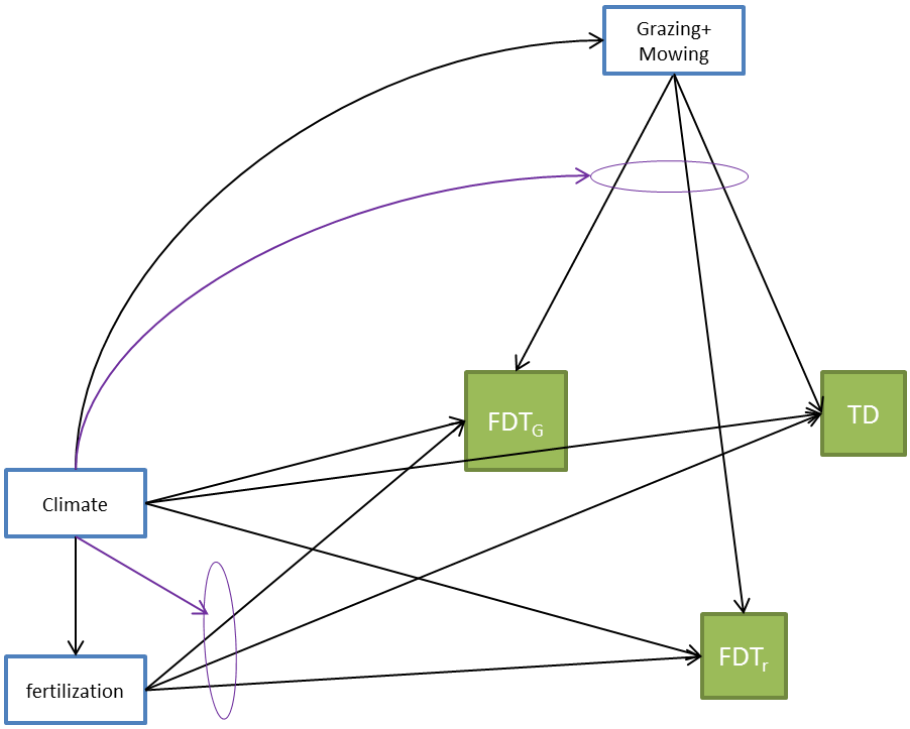

Figure 6: Conceptual schema based on hypotheses on the effect of climate and management on functional diversity. FDTG: Functional diversity of trait linked to growth; FDTR: Functional diversity of traits linked to reproduction; TD: Taxonomical diversity. In purple, the effect of climate on the effects of management on functional diversity (i.e. conditional effects)

The effects of management on functional diversity **differ regarding climatic condition** (Vesk *et al.*, 2004; de Bello *et al.*, 2005a, 2006). The effect of management is so conditioned by the climatic conditions (conditional effect). Few studies were made with a large scale to be able to test and separate the effect of climate and management (Michaud *et al.*, 2012b).

V. Objectives, hypotheses, and research strategy

The overall objective of this PhD program is to assess the effects of management and climate on the plant functional diversity related to ecosystem services of permanent grasslands. This objective is thought as a step forward to the evaluation of ecosystem services taking into account management, climate and their effects on plant functional diversity.
The figure 7 presented the combination of the conceptual model presented in figure 5 and 6. The objective of this PhD is to test this conceptual model.

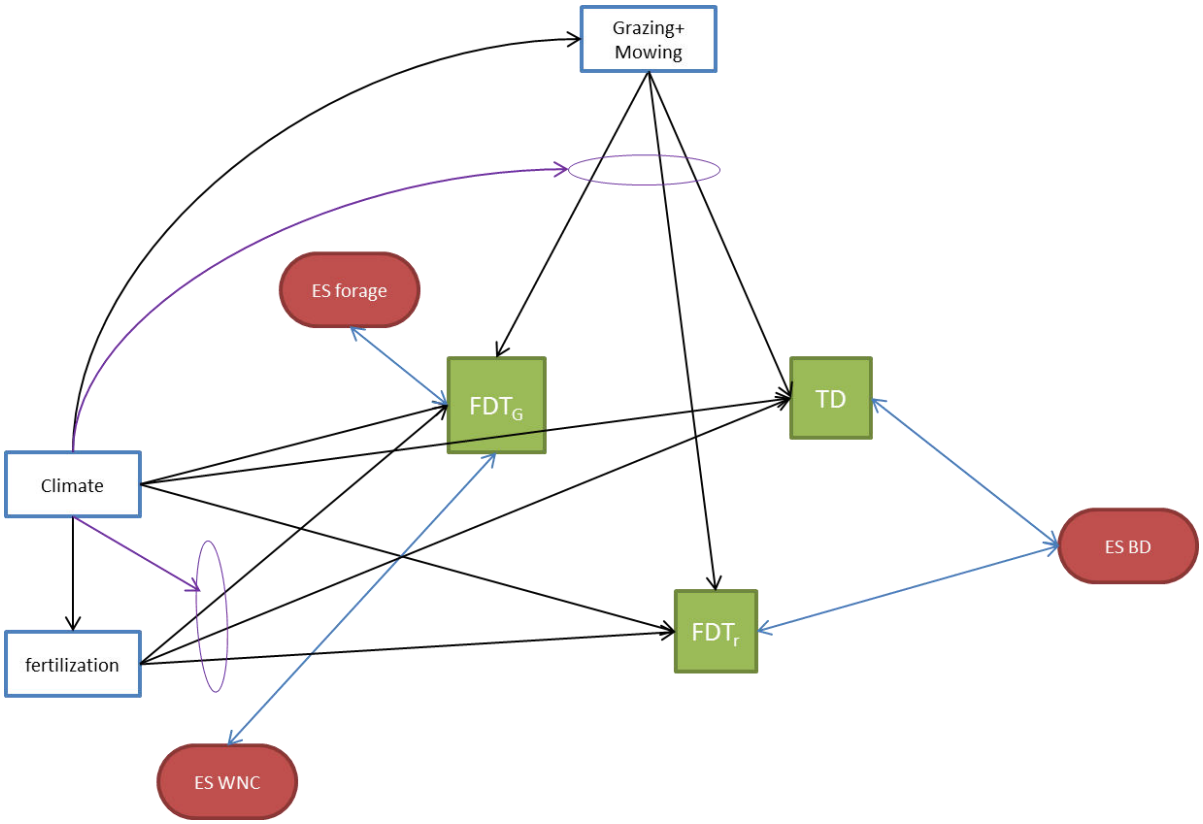

Figure.7: conceptual model of the hypothesis on the relationships between ecosystem services, functional diversity, management and climate. ESFORAGE: Ecosystem services related to forage production. ESWNC: Ecosystem service related to the nitrogen, carbon and water cycle. ESBD: Ecosystem services related to biodiversity conservation. The blue arrow represented the links between ecosystem services and functional diversity. FDTG: Functional diversity of trait linked to growth; FDTR: Functional diversity of traits linked to reproduction; TD: Taxonomical diversity. In purple, the effect of climate on the effects of management on functional diversity (i.e. conditional effects).

To achieve this overall objective, the steps of the research were to:

1. **Select functional diversity criteria that could be relevant to evaluate ecosystem services.** This selection was based on literature review and expert interviews. This objective is based on the hypothesis that consensual functional diversity criteria related to ecosystem services can be found. This selection is presented in the first chapter.
2. **Assess the possibilities and limits of calculating the functional diversity criteria** with functional trait values extracted from functional trait databases. We tested the hypothesis that the errors induced by the use of functional trait databases instead of measured values do not impede the calculation of functional diversity. In the second chapter, we evaluated different of these errors.
3. **Evaluate the effects of management and climate on the selected functional diversity criteria.** This step was based on the analysis of a database regrouping survey of 500 grasslands in France and Switzerland. We tested the hypothesis that functional diversity of grassland is largely determined by climate and management within the range of grasslands available in the database. In the third chapter, different analyses were made to evaluate the effects of management and climate on the functional diversity criteria.

Chapter 1: Selection of functional diversity criteria linked to grassland ecosystem services

Abstract Chapter 1

The objective of this chapter is to propose a list of functional diversity criteria linked to ecosystem services. Eight ecosystem services were studied: quantity of forage, forage quality, stability of the forage production, biodiversity conservation, pollination, carbon sequestration, drought resistance and nitrogen fertility. The different services were then subdivided into ecosystem functions, processes and states. Interviews of experts and a review of the scientific literature were used to selected functional diversity criteria related to these ecosystem processes. Twenty-nine functional diversity criteria were selected. Levels of confidence of the relationships between functional diversity criteria and ecosystem processes were extrapolated from the interviews. For the carbon sequestration, nitrogen fertility, drought resistance and t forage services, the functional diversity criteria that were selected are mainly related to the leaf economic spectrum. For biodiversity and the pollination, the selected functional diversity criteria are mainly based on the flower characteristics and the structure of the vegetal cover.

I. Objective

The objective of this chapter is to propose a list of functional diversity criteria related to ecosystem services delivered by permanent grasslands. These functional diversity criteria could be potentially used to evaluate the provision of ecosystem services. This list of criteria was used in the rest of the work.
To reach this objective, the first step was to select the most relevant ecosystem services provided by permanent grasslands and define ways to quantify them. The second step was to identify the processes and functions linked to these ecosystem services. The third part was to propose a list of functional diversity criteria related to these processes and functions. These links were assessed by literature survey and experts' interviews. The two last parts of the chapter mainly rely on the works of Marine Benoiste (2nd year of Master EBE; AgroParisTech, France), Tiphaine Audic (2nd year of Agronomic school; ENSAT, France and Switzerland) and Dr Rosalinde Van Couwenberghe (Post Doc, LAE, France).
In this chapter, a functional diversity criterion is defined as one variable that evaluate one aspect of functional diversity. These functional diversity criteria could be *census stricto* functional diversity variable (index calculated from functional traits) or some other variables used as proxy. Indeed, sometimes the trait values of a functional trait may sometimes be difficult to obtain so that a proxy has to be used. Taxonomical and phylogenetic diversity can sometimes be used as proxy of functional diversity. For example, the relative abundance of legumes is a good proxy of the relative abundance of N_2 fixing individuals.

II. Selection of the ecosystem services

A. Choice of the ecosystem services for the study

The selection of ecosystem services was made by elimination. We started from the list of ecosystem services proposed by Le Roux *et al.*, (2008) for agro ecosystems, merging from the report of a large panel of French experts in "agriculture and biodiversity". This list was adapted from Millennium Ecosystem Assessment list (MEA, 2003). In this list, ecosystem services were split in 3 categories: provisioning services, supporting services and regulating and cultural services (Figure1.1). Provisioning services are the services that contribute to the revenue of the farmer (animal and vegetal production). Environmental services (regulating and cultural) did not contribute directly to the farmer revenue but important for society. Supporting services contributed to the stability of the functioning of the ecosystem so to the provision of the others services.
We made different selections regarding different principles. The selections were based on literature and also discussed during meetings with researchers of the two research teams (LAE INRA-UDL and Agroscope) and was validated by the consortium of the FP7 MULTISWARD project during the second general meeting of the project (Paris, France February 2011). These ecosystem services were also considered as important services of the grasslands in Europe by a panel of stakeholders (Van den Pol-van Dasselaar *et al.*, 2012).
Our first selection was to eliminate the ecosystem services that were not necessarily concerned the permanent grassland in the North West of Europe. Fire regulation is an important of ecosystem services in the Mediterranean region. The Mediterranean region was out the focus of the MULTISWARD project. For the other European regions, the fire regulation of ecosystem is not really important. We so deleted the fire regulation in our list. For the same reason, we eliminated water quality in terms' of pesticide and herbicide pollution. The use of herbicide and pesticides on permanent grasslands is very low. The pollution by pesticide and herbicides is not really a problem for the grasslands.

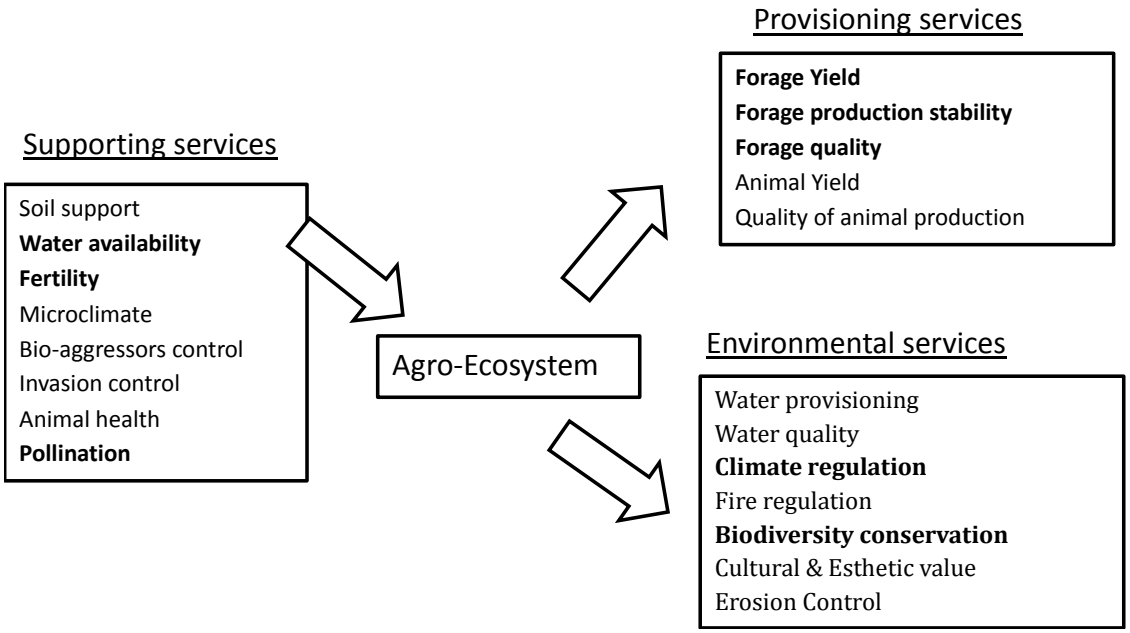

Figure1. 1 List of ecosystem services for agroecosystems adapted from le Roux *et al*.,(2008). In bold, the ecosystem services we studied in this work.

Secondly, we only considered the grassland plot individually i.e. we did not study the ecosystem services that grasslands can provide for other types of ecosystems and the ecosystem services that can be provided from several plots.

We eliminated the control of pest diseases and the microclimate that can be services provided by grassland to other types of ecosystems. The different services related to the animal (animal production in quantity and quality and the animal's health) rely on the set of grassland plots in a farm. Thus, we eliminated the ecosystem services based on animal production.

Finally, we eliminated ecosystem services with an absence or unknown link with biodiversity. Some ecosystem processes are more related to the absence or presence of vegetal cover all during the season than to difference in term of botanical composition. Erosion is one example of these processes more that is important under crops than under permanent grasslands (Cerdan *et al.*, 2010). We did not find any study that shows differences in term of erosion between grassland with different vegetal diversity. We so eliminated the erosion. We eliminated for the same reason the nitrate leaching, soil support and water provisioning.

For the esthetic value of grassland, some relationships can be made with some characteristic of the vegetation and the esthetic value. However, the esthetical value of grassland is quite subjective.

For the control of invasive species, the results are very dependent on the ecology of invasive species and general trends are hard to evaluate.

Eight different ecosystem services were selected based on their relevance with respect to permanent grasslands and further studied (Table 1.1).

We studied three provisioning services (services that contribute to the production of marketable goods and to the revenue of the farmer): **forage yield, forage quality and forage production stability**. Three supporting services (services that contribute to the stability of the functioning of the ecosystem and thus to the provision of the others services) were also studied: **pollination, nitrogen fertility and dryness resistance.** Two environmental services (services that do not contribute directly to the farmer revenue but are important for the society) completed the set of selected services: **carbon sequestration and biodiversity conservation**.

Table.1.1: Ecosystem Services selected

Supporting services	Drought resistance
	Nitrogen Fertility
	Pollination
Regulating and cultural services	Carbon sequestration
	Biodiversity conservation
Provisioning services	Forage production stability
	Forage production
	Forage quality

B. Description of the selected ecosystem services and their quantification

1. Biodiversity conservation and pollination

Biodiversity conservation is a wide and fuzzy concept. It can only be assessed partially. We proposed to study different taxa (plant and animals). The conservation of six taxa was used to describe the conservation of animal taxa: earthworms, spiders, orthoptera, honeybees, bumblebees and butterflies. They were chosen through a literature review, meetings with experts and based on four principles.

-These taxa were first selected regarding their relationships with grassland flora.

-These taxa had different spatial distributions in grassland: endogenous (living underground), epigenous (living on the surface of the soil under the grass layer) or exogenous (living in air).

-The taxa had to mostly live within the grassland ecosystem (travel only small distances) and then to be very dependent on the grassland.

- Per food chain level, one taxa was selected and these taxa were belonging only to one trophic level. For the detritivores, **earthworms** were preferred to nematodes because they are present at a single trophic level (Wardle *et al.*, 2003). For herbivores, we chose the **orthoptera**, but only crickets and locusts (Marini *et al.*, 2008). For the predators group, we chose the **spiders** for their wide distribution in the micro- and macro-space (Dennis *et al.*, 1998; Jeanneret *et al.*, 2006). For the pollinators, we chose three taxa: **honeybees, bumblebees and butterflies**, because they are foraging different flowers and may respond differently to the vegetation criteria. Furthermore, the three taxa were also used to evaluate the pollination service. For the primary producer, the **herbaceous angiosperm plants** were selected.

For each taxon, the conservation of its biodiversity was assessed by the **abundance** (number of individuals) and the **diversity** (number of species) except for the earthworms, plants and honeybees. For the earthworms, we only considered the abundance and distinguished the abundance of the anecic, epigenous and endogenous earthworms. As the domestic bee is a single species, only its abundance was evaluated. For spiders, a separation was made between web spiders and soil spiders. For plants, we assessed the species richness of plants using directly the number of plant species.

Pollination could be measured by the number of flowers that are transformed into a fruit. However, this information is hard to obtain and the abundance of pollinators was used to assess the pollination services, using the same criteria selected for the biodiversity conservation of these taxa.

2. Ecosystem services relied on biogeochemical cycles

Nitrogen fertility is generally defined by the nitrogen quantity available in the soil. However, this quantity of nitrogen may be provided from high fertilizer input. Only the nitrogen originating from the ecosystem should be considered for the evaluation of the fertility provided by the ecosystem. We proposed to evaluate the fertility by the difference between the **quantities of nitrogen uptake by the plant minus the quantity of nitrogen fertilizer**.
Drought resistance is the capacity of the vegetation to resist to the water limitation. The resistance is due to the ability of the plant to take up water from the soil and to limit its transpiration. The temporal aspect is also important for the drought resistance. We proposed to assess the dryness **as the number of days where the photosynthesis is limited by water limitation**.
We chose to define carbon sequestration by the dynamics of the carbon in the soil.

3. Forage production ecosystem services

Forage production is generally quantified by the **annual biomass yield**. Dry matter biomass is generally used and reported by area unit. Forage production is generally measured in t DM ha^{-1} $year^{-1}$.
Forage quality can be measured by many different aspects. One of the main criteria to assess forage quality in ruminant nutrition is the concentration and availability of energy in the forage, which were the criteria retained for this study. We chose to use **the digestibility of the organic matter, the nitrogen content and the congestion of the** forage to estimate the energetic quality of the forage, by analogy with Baumont *et al*. (2012).
Forage stability depends on the biomass seasonal stability (stability of the yield) but also on the stability of the forage quality descriptors.

Table.1 2 Ecosystem: services and their measurements

Services	Measurements
Carbon sequestration	Increase of soil carbon
Biodiversity conservation	Abundance of 6 taxa (earthworms, domestic bee, bumblebees, butterflies, orthoptera , spiders,) Specific richness of 5 taxa(plants, bumblebees, butterflies ,orthoptera , spiders)
Nitrogen fertility	Nitrogen uptake- Nitrogen fertilization
Pollination	Abundance of 3 taxa (,domestic bee, bumblebees, butterfly)
Dryness resistance	Number of days of water limitation of the photosynthesis
Forage production	Yearly biomass production
Forage quality	Digestibility of organic matter Nitrogen content Congestion
Forage stability	Biomass seasonal stability Nutrient seasonal stability

III. Subdivision of the ecosystem services into ecosystem functions, processes and states

The subdivision of the ecosystem services into ecosystem functions, processes and states in the literature is variable between studies so we chose our own. We choose the way to subdivide the different ecosystem services in consultation with the experts (Table.1.3) and the literature.

A. Biodiversity conservation and pollination

The abundance of animal taxa generally relies on the **quantity of available food** and on the **quality of the habitat**. Sometimes, the abundance is also related to the diversity of the food (example the abundance of domestic bee partly depends on the diversity of pollen). For the spiders, the quantity of food is related to the abundance of orthoptera. The species diversity of animal taxa also relies on **the food and the habitat**, but more on the diversity of food source and the diversity of habitats. The spider's diversity (soil and web) relies only on the habitat.
For the plant diversity, we directly used the number of plant species.

B. Carbon sequestration, Nitrogen fertility and Dryness resistance

Carbon sequestration is related to the **carbon cycle** between the soil and the plant. We propose to model the carbon cycle with 7 pools and 14 processes (Figure.1.3). Atmospheric carbon is captured by plants through photosynthesis. This carbon is then allocated either to the aboveground part or to the belowground part (allocation). Carbon in the leaves may be lost through harvesting (cut, pasture), aboveground respiration or aboveground senescence. Carbon of dead aboveground part goes to the above-ground residues pool. Carbon in the belowground part may be lost by roots respiration, roots senescence and by rhizodeposition. Carbon of dead roots goes to the belowground residues. The rhizodeposition go directly from carbon roots to one of the carbon soil pool. Carbon of the two residues pools is decomposed in to three pools of organic matter (active, slow and passive pool). The time of residence of the carbon is the main difference between the different pools. The transfers between soil pools depend on the decomposition speed and the repartition of the residue between the pools. Carbon is lost from the different soil pools by respiration.
Nitrogen fertility relies on the **nitrogen cycle** in the plant and in the soil (Figure.1.4). For the plant part, nitrogen cycle is close the same that the carbon cycle. Nitrogen in the aboveground part may be lost by a cut or pasture (plant export) and by aboveground senescence. During senescence, one part of the nitrogen in the leaves may be reused (remobilization): same for the root's senescence. Decomposition of the residues is transferred in three different soil organic matter pool depending of the speed decomposition and the repartition of the decomposition between the different soil pools. Nitrogen is after mineralized into ammonium (Mineralization). Ammonium may be lost by runoff and volatilization. Ammonium is transformed into nitrate in the soil (nitrification). Nitrates may also be lost by leaching or by denitrification. Plants may uptake from the soil nitrates and ammonium (plant uptake). Plant uptake depends of the preference of the species for the ammonium or the nitrates and the quantity of water uptake. One part of the nitrogen in the roots provide also from the symbiotic fixation of legumes. Nitrogen of the roots is transferred into the aboveground part (plant nitrogen transfer).
The drought resistance is related to the **water cycle** in grassland. The modeling of the cycle is presented in Figure.1.5.
Rainfall is the input of water in to the ecosystem. One part of the rainfall is intercepted by the leaves surface (interception). One part of this water is evaporated (leaves evaporation), the rest of the water on the leaves drip on the surface water supply (dripping). One part of the surface water supply is infiltrated into the different soil water pool (infiltration). The rest is lost by runoff or evaporation. The soil water is divided in three pools regarding the water potential (low water potential, medium water potential and high water potential). Each supply had a maximum capacity. The water provided from the infiltration goes first in high water potential then in medium water potential and to finish low water potential. Some water is lost by percolation. The plants may uptake water from the low and medium water potential. The plant uptake depends on the plant transpiration. Some water from the leaves is lost by transpiration introducing a water

imbalance in the leaves. The balance is made by a transfer of water from the roots to the leaves (water transfer). Root water balance is maintained by the water in the soil. If the quantity of water in the soil is too low, the transpiration stops and so the photosynthesis. Quantity of water that a plant may uptake (plant uptake).

C. Forage production, quality and stability

In the literature, the links between forage services and vegetation are directly made. We choose to directly link the services to functional diversity criteria.

IV. Links between processes and functional diversity criteria

A. Methods

The assessment of the links between the different processes and the functional diversity criteria is based on **a review of bibliography and expert interviews**. The list of the experts is presented in Table 1.3.
The interviews were conducted by Marine Benoiste (biodiversity), by Tiphaine Audic (biogeochemical services) and by Dr. Rosalinde van Couwerberge (forage). The literature review was performed by the same persons, as well as by Prof. Sylvain Plantureux and Dr. Bertrand Dumont for the conservation of biodiversity.
The objectives of the interviews were not only to select functional diversity criteria related to ecosystem services. Another objective of the interviews was to develop the ecosystem services tools based on the experts. Only the information on biodiversity was used to developed indicators in the MULTISWARD project. At least, two experts were chose for each ecosystem services. The selection was based on the network of researchers in France, Switzerland, Norway and Germany.
The interviews were conducted the same way. The first step was to present a quick review of the literature. We presented during the interview the decomposition of the ecosystem services and the first results obtain from the literature. The experts were used to approve the finding of the literature review and to obtain missing information. At the end of each interview, we update our results for the next interview.
We added some time to transform the proposition of the experts. Sometimes, the criterion proposed by the experts was not available (too few information in the functional trait database). In this case, we used proxy. Furthermore, some information from the experts was not defined as functional diversity criteria. For example, the trade-off between acquisition and conservation of nutrients was often used as an indicator of several processes. We chose the community weighed of leaf dry matter content.
For each link, we also judged the quality of the links between the functional diversity and the processes. The quality was judged regarding the level of confidence of the experts during the interview and the quality of information found in the bibliography.

B. Results

The list of the links between the different processes and the functional diversity criteria is presented in the Tables 1.8 to 1.10 for the biodiversity services, in the Tables 1.5 to 1.7 for the biochemical environmental services, and in Table.1.11 for the forage. The bibliographic references and the experts are given in these tables.

Table.1.3: List of the experts interviewed for the selection of functional diversity criteria with their organization and the services of their expertise.

Expert name	Organization	Services
Servane Lemauviel-Lavenant	Université de Caen - Basse Normandie France	Carbon and nitrogen cycles
Jean-Bernard Cliquet		
Emmanuelle Personneni		
Frédérique Louault	INRA Clermont-Ferrand France	Carbon and nitrogen cycles
Catherine Roumet	CNRS Montpellier (CEFE) France	Carbon, nitrogen and water cycles
Sandra Lavorel	CNRS Grenoble (LECA) France	Carbon and nitrogen cycles
Wolfgang Wilcke	Institute of Geography (GIUB) Germany	Carbon and nitrogen cycles
Yvonne Oelmann		Carbon and nitrogen cycles
Lutz Merbold	ETH Zürich Switzerland	Carbon, nitrogen and water cycles
Jens Leifeld	Agroscope Reckenholz-Tänikon (ART) Switzerland	Soil organic matter carbon
Hansruedi Oberholzer		Soil processes
Pierluigi Calanca		Water cycle in general
Thomas Walter		Butterflies/Orthoptera
Philippe Jeanneret		Spiders/Butterflies
Anne Farugia	INRA Clermont-Ferrand France	Bumblebees
Bertrand Dumont		Bumblebees
Stein Joar Hegland	Sogn og Fjordane University college Norway	Bumblebees/ Honeybees
Reidum Pommeresche	Bioforsk Norway	Spiders/ Earthworms
Daniel Cluzeau	Université de Rennes I France	Earthworms
Axel Decourtye	Acta Paris France	Honeybees
Philippe Bachelard	Natural History Society Alcide-Orbigny France	Butterflies
René Baumont	INRA Clermont-Ferrand France	Forage services
Sylvain Plantureux	Université de Lorraine France	Forage services

1. Carbon sequestration, nitrogen fertility and dryness resistance

Table.1.5 presents the links between the ecosystem processes related to the carbon sequestration and the functional diversity criteria. All the criteria are **functional identity criteria (community weighted mean values).** The most frequent functional traits are leaf economy spectrum traits (LNC and LDMC). These functional traits were proposed as indicator of the acquisition/conservation trade-off. For example, the leaf nitrogen content was proposed as an indicator of the nitrogen content in the roots. The

community weighted mean value of the maximal vegetative height was proposed as indicator of the vegetation height at the defoliation events. Onset of flowering is used as an indicator of the phenological cycle of the plants and so of the leaves life span. It is interesting to note that carbon soil processes were not identified as directly influenced by the vegetation.
Table.1.6 presents the links between the ecosystem processes related to the nitrogen fertility and the functional diversity criteria. The important functional diversity criteria are the same as for the carbon sequestration. Nitrogen and carbon cycles are strongly interconnected in grassland ecosystems. The main difference between the two cycles for the plant is the uptake. For the nitrogen uptake, one part derives from the symbiotic N2 fixation. The percentage of legumes is generally used to evaluate symbiotic N2 fixation. For the uptake of nitrogen from the soil, the factor identified as most important factor was the water flow from the soil (see Table.1.7). The "preference" of the species for a form of nitrogen is also related to the leaf economics spectrum. Soil processes were for the majority identified as not directly influenced by the vegetation expect for ammonification. Ammonification is influenced by the ratio between soil fungi/bacteria. This ratio is related to the leaf economics spectrum.
For the dryness resistance, the links between water ecosystem process and functional diversity is presented in Table.1.7. The LAI was identified as a key component in the water cycle (transpiration, rainfall interception, evaporation). LAI may be related to standing biomass and so to the community weighted mean value of the vegetative height. The leaf angle is also a key factor on the ratio between dripping/ leaves evaporation. Roots traits are also important for infiltration and the water uptake. However the roots trait information is very lacunar and some proxy have to be used. The percentage of forbs was proposed as an indicator of the roots size. These two relationships are strongly hypothetical and no based on known results.

2. Biodiversity conservation services

Links between the animal's conservation and plant functional diversity are presented in Table.1.8 for the spiders and the orthoptera; in Table 1.9 for pollinator taxa (butterflies, bumblebees, domestic bee) and in Table.1.10 for earthworms. The habitat of the different taxa relies mainly on the structure of the vegetation (height and heterogeneity of the vegetation). We used the functional trait maximum vegetative height to assess the structure of the vegetation (CWMH for the height and functional dispersion of vegetal height for the heterogeneity). The relationships between vegetation structure and abundance/diversity differ between the taxa. For the bumblebees, the relationships follow a humped-shave curve with an optimal between 7 and 20 cm. At the difference for the butterflies, the relationship is linear and positive. The habitat relied also for some taxa on specific characteristic such as the temperature for the orthoptera, the percentage of apiaceous for the web spiders, the thickness and heterogeneity of litter for the orthoptera (only for diversity) and bumblebees. For the pollinator's taxa, the food relies mainly on relative abundance of species that the taxa may feed on for the quantity of food and the number of species that the taxa can feed on. For the orthoptera, the food relies on the biomass and the diversity of leaves characteristic (assessed here with the number of plant species). The food for spiders depends on the abundance of orthoptera and so on the same criteria. For the earthworms depending of their functional groups, the food relies on the quality and the quantity of litter and/ or the food from the roots.

3. Forage ecosystem services

The leaf economics spectrum traits were identified as good indicators of the different forages services (Table.1.11). Percentages of grass, legumes and diverse are also useful for the evaluation of forage ecosystem services.

V. Chapter Conclusion

Table.1.4 presents the summary of all the different functional diversity criteria selected in this chapter with the number of services the criterion has been identified as strongly relevant. Twenty-nine different functional diversity criteria were selected.
Among these twenty-nine criteria, ten are based on the relative abundance of plant groups (functional or taxonomical groups). The general separation between grasses, legumes and diverses is used in all the types of services. For the pollinator species, the relative abundance of the plant species that are used as a source of food is a key factor. Four criteria are based on the number of species in one plant group (the number of species and three for the species used as food source by the pollinators).
For the criteria based on functional traits, the community weighted mean value is the aspect of functional diversity most frequently identified as important for the service. Five different community weighted mean values were used (vegetative height, leaf dry matter content, leaf nitrogen content, onset of flowering and specific leaf area). The CWMH and CWMLNC are the ones identified as important for the largest number of services (17). The CWMLDMC was also identified as important for a lot of services (11). For the other functional diversity components, functional richness was chosen 2 times, functional dispersion 2 times and functional evenness only one time. Several studies showed that functional identity is more related to ecosystem services than functional diversity (Mokany *et al.*, 2008; Klumpp and Soussana, 2009; Lavorel *et al.*, 2011; Lavorel and Grigulis, 2012) .
This list of criteria was afterwards used throughout the work. In the second chapter we assessed the possibility to calculate these criteria using functional trait databases.

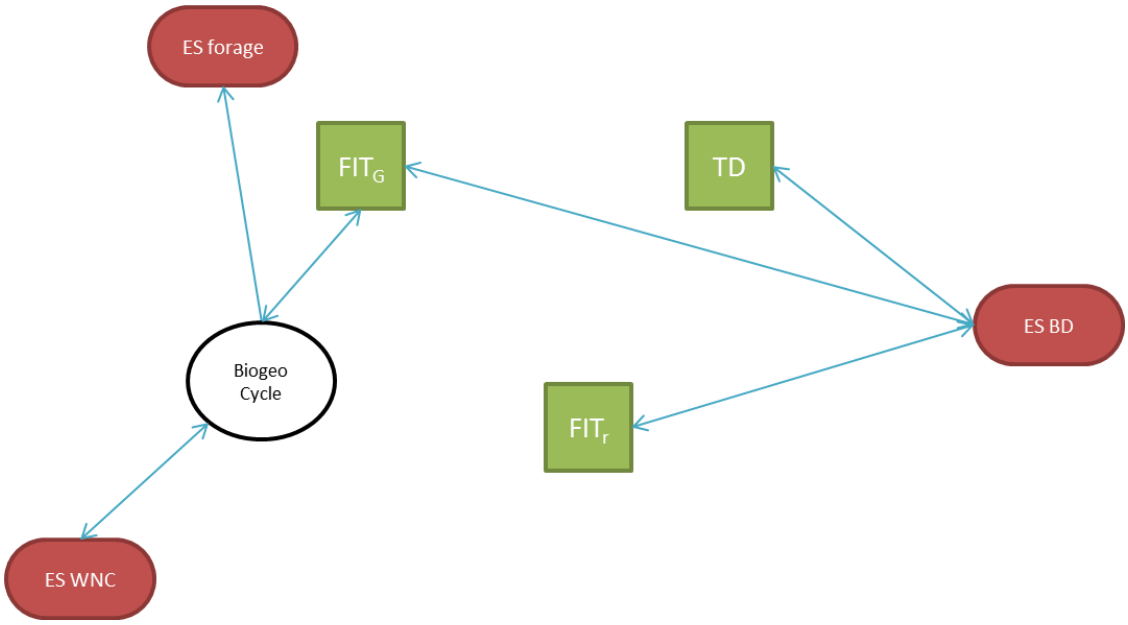

Figure1.2: Modification of the conceptual model regarding the outcome of the first chapter. FIT$_G$: Functional identity of traits linked to the growth; FIT$_R$: Functional identity of traits linked to the reproduction; TD: Taxonomical diversity; ESFORAGE: Ecosystem services related to forage production; ESWNC: Ecosystem service related to the nitrogen, carbon and water cycle
ESBD: Ecosystem services related to biodiversity conservation.

Regarding the results of the interviews, we can update the conceptual model we propose in the introduction. The functional diversity was mainly functional identity. Very few criteria based on other functional diversity component like functional dispersion or functional richness. In the conceptual model; we specified that the different functional diversity criteria were functional identity ones. The functional traits related to the vegetative growth (specific leaf area, leaf dry matter content, grass percentage…) were

link to the biogeochemical cycles and the ecosystem services depending on theses cycles. The taxonomical diversity and functional identity of the reproductive traits were related to the conservation of biodiversity. The traits related to growth were also related to the conservation of biodiversity.
The study between the different functional criteria could be interesting to test the potential trade-off between ecosystem services. However as for the study of the effect of management and climate on these criteria, it would be interesting to know the accuracy of these functional diversity criteria. Indeed, if a functional diversity criterion is not well measured, its relation with the other criteria would not be accurate.

Table.1 4: List of Functional diversity criteria, their description, abbreviation and the number of processes, for which the criterion has been identified as strongly relevant.

abbreviation	**Description**	**Number of process**
% forbs	percentage of forbs	1
% Grass	percentage of grass	4
% legumes	percentage of legumes	7
Legumes/grass	ratio legumes/ grass	1
% apiaceous	percentage of apiaceous	2
% dicot	percentage of dicots	1
% Bee sp	percentage of species with bee pollination	1
%Bumblebeessp	percentage of species with bumblebee pollination	1
% Butterflysp	percentage of species with butterfly pollination	1
% Bumblebeessp legumes	percentage of legumes with bumblebee pollination	1
Beenbsp	number of plant species visited by bee	1
Bumblebeesnbsp	number of plant species visited by bumblebee	1
Butterflynbsp	number of plant species visited by butterflies	1
Number sp	number of plant species (herbaceous)	2
CWMH	community weighed mean value of vegetative height	17
CWMLDMC	community weighed mean value of LDMC	11
CWMLNC	community weighed mean value of LNC	17
CWMOFL	community weighed mean value of OFL	6
CWMSLA	community weighed mean value of SLA	3
FDH	functional dispersion of vegetative height	5
FDSLA	functional dispersion of SLA	1
FEH	functional evenness of vegetative height	2
FRH	functional richness of vegetative height	2
FRLDMC	functional richness of LDMC	2
Ellenberg Temperature	Ellenberg index of temperature based on dominance	2
Humidity Ellenberg	Ellenberg index of humidity based on dominance	1
Flower duration	number of months with the presence of flower	3
Nectar quantity	community weighed mean value of Nectar quantity	1
Pollen Quantity	community weighed mean value of Pollen quantity	1

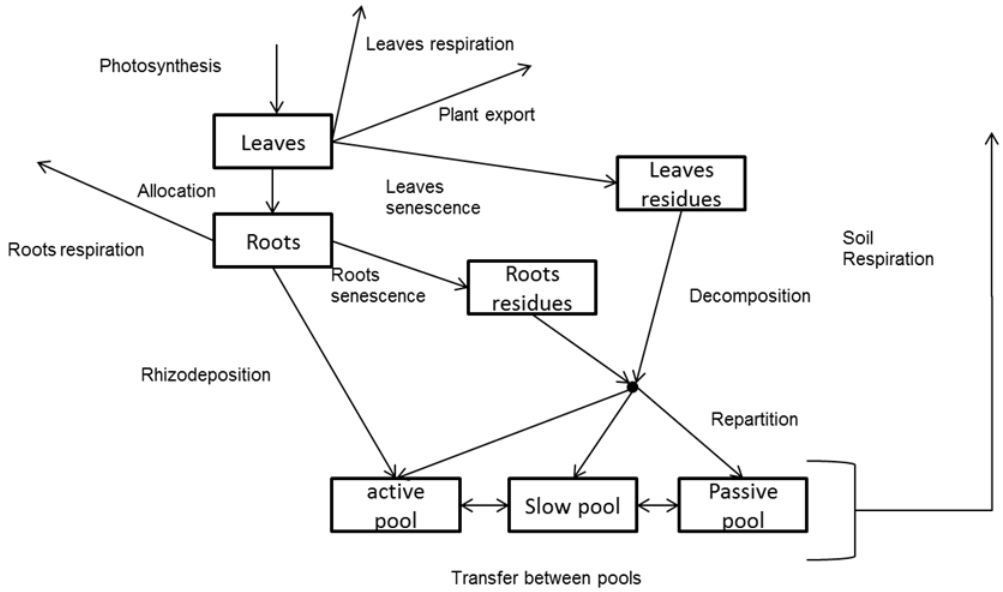

Figure.1.3: Conceptual model of carbon cycle in grassland used during the interview.

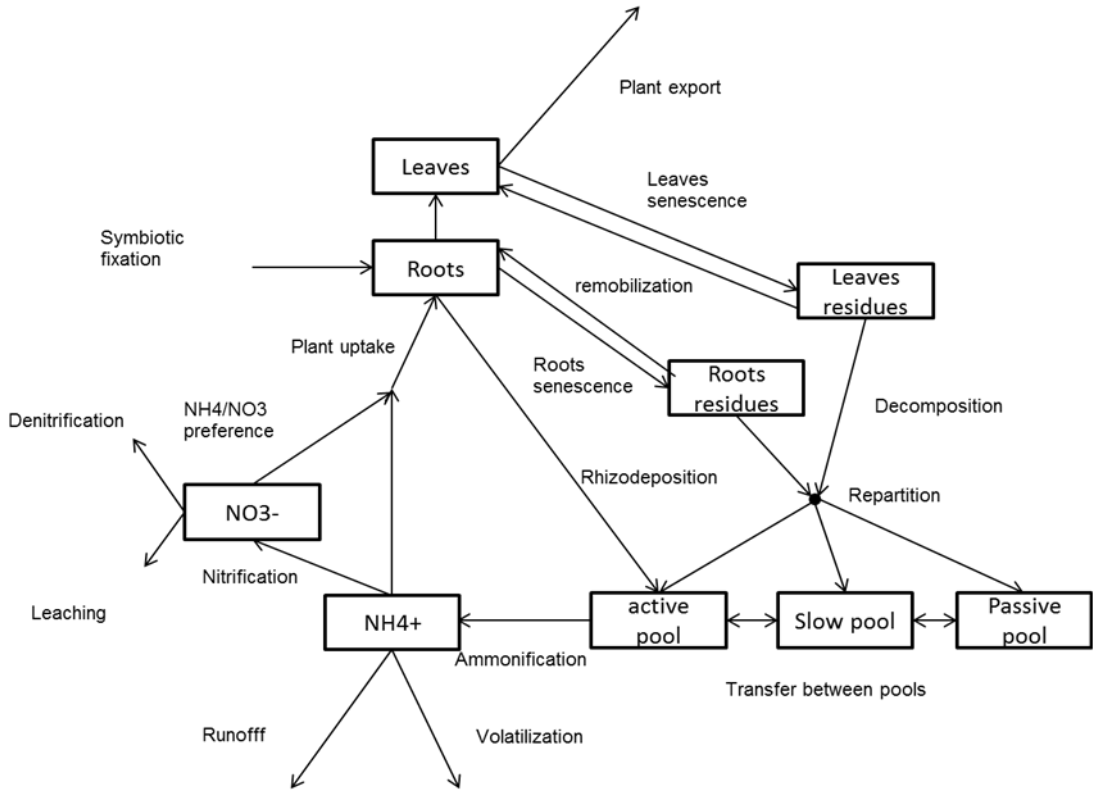

Figure.1.4: Conceptual model of nitrogen cycle in grassland used during the interview.

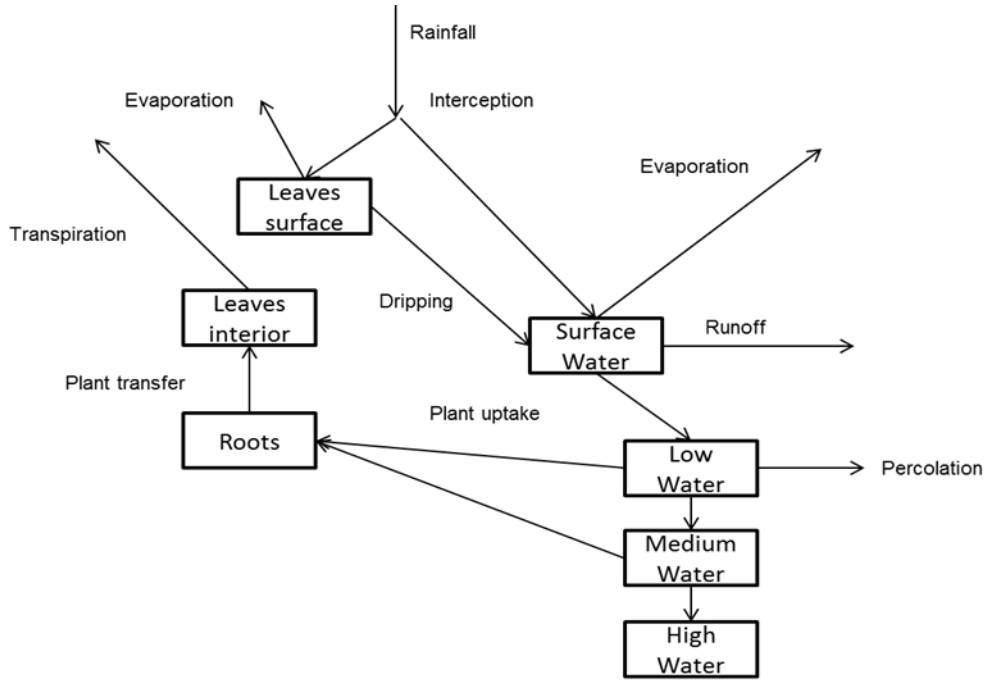

Figure.1.5 : Conceptual model of water cycle in grassland used during the interview.

Table 1.5: Relationships between functional diversity criteria and the ecosystem process/ state linked to the carbon sequestration. The confidence of each relationships was assessed based on the experts and literatures surveys.(CWM community weighed mean value, LNC leaf nitrogen content , LDMC leaf dry matter content, OFL onset of flowering and H : maximal vegetative height).Direction of the relationships (positive +, negative: -). Confidence on the links between FD and the process (strong +, average=; weak -).

Services	Function	Process	FD Criteria	FD Criteria used	Direction Relationships	Confidence	Reference
C sequestration		Photosynthesis	Photosynthetic rate	CWMLNC	+	+	Wright *et al.* (2004); expert agreement
	Plant Growth	Leaves Allocation	Plant economic spectrum	CWMLDMC	-	-	S. Lemauviel; L.Merbold; C. Roumet
		Plant Export	Vegetative height	CWMH	+	=	S Lavorel
		Leaves respiration	Metabolism rate	CWMLNC	+	+	(Reich *et al.*, 2008) ; C. Roumet
		Roots respiration	Root Nitrogen content	CWMLNC	+	+	(Bahn *et al.*, 2006; Reich *et al.*, 2008); C. Roumet
		Rhizodeposition	Root Nitrogen content	CWMLNC	+	=	(Nguyen, 2003; Lesuffleur, 2007) ;C. Roumet (Perez-Ramos *et al.*, 2012).
		Leaves senescence	Leaf ages	CWMOFL	-	-	(Guiboileau *et al.*, 2010)
	Plant carbon lost	Root senescence	Equilibrium between roots and leaves biomass	CWMH/ CWMOFL		-	Bingham (2012b); E. Personeni; F. Louault; (Bingham, 2012a)
		Speed decomposition	lignin/ nitrogen	CWMLNC	+	+	S. Lavorel; (Parton *et al.*, 1988; Freschet *et al.*, 2012)
	Carbon residue decomposition	Repartition between the soil organic matter pool	Chemical composition of leaves	CWMLDMC	+	=	E Personneni
		Transfer between pool		No direct effect of the vegetation		=	S. Lavorel; Y. Oelmann
	Soil decomposition	Soil respiration	Soil C/N ratio	No direct effect of the vegetation		-	H. Oberholzer; J. Leifeld

Table 1 6: Relationships between functional diversity criteria and the ecosystem process/ state linked to nitrogen fertility. The confidence of each relationships was assessed based on the experts and literatures surveys.(CWM community weighed mean value, LNC leaf nitrogen content , LDMC leaf dry matter content, OFL onset of flowering and H : maximal vegetative height). Direction of the relationships (positive +, negative: -). Confidence on the links between FD and the process (strong +, average=; weak -).

Services	Function	Process	FD Criteria	FD Criteria used	Direction relationship	Confidence	Reference
N Fertility		Plant Export	Vegetative height	CWMH	+	=	S Lavorel
		Leaves senescence	Leaves ages	CWMOFL	-	-	Guiboileau *et al.* (2010)
		Remobilization	Leaf nitrogen availability	CWMLNC	-	=	Bingham (2012b); E. Personeni; F. Louault
	Plant nitrogen loses	Roots senescence	Equilibrium between roots and leaves biomass	CWMH/ CWMOFL	-	-	Experts' agreement) (Eckstein *et al.*, 2002).
		Speed decomposition	lignin/ nitrogen	CWMLNC	+	+	(S. Lavorel; (Parton *et al.*, 1988; Freschet *et al.*, 2012))
	nitrogen residue decomposition	Repartition between the soil organic matter pool	Chemical composition of leaves	CWMLDMC	+	=	E Personneni
		Transfer between pool		No direct effect of the vegetation		=	E Personneni
		Ammonification	Fungi/bacteria	CWMLDMC	-	-	(Grigulis *et al.*, 2013), S. Lavorel).
		runoff		No direct effect of the vegetation		=	Y. Oelmann
		Volatilization		No direct effect of the vegetation		=	Y. Oelmann
		Nitrification		No direct effect of the vegetation		=	Y. Oelmann
		Nitrate leaching		No direct effect of the vegetation		=	
	soil process	Denitrification		No direct effect of the vegetation		=	Y. Oelmann
		preference Ammonium/Nitrate	Acquisition/ conservation trade off	CWMLDMC		=	S. Lemauviel ; (Bardgett *et al.*, 2002; Weigelt *et al.*, 2005)
	Plant Uptake	Water flux	Plant Water uptake	FRH/FEH/CWMSLA	+	=	
	Symbiotic fixation	Symbiotic fixation	% of legumes	% of legumes	+	+	(Høgh-Jensen *et al.*, 2004; Carlsson *et al.*, 2009; Soussana and Tallec, 2010)
	Plant nitrogen transfer	Nitrogen transfer	Nitrogen leaves metabolism	CWMLNC	+	-	

Table.1.7: Relationships between functional diversity criteria and the ecosystem process/ state linked to dryness resistance. The confidence of each relationship was assessed based on the experts and literature surveys (CWM community weighed mean value, FR functional richness, FE functional evenness, SLA specific leaf area, OFL onset of flowering and H: maximal vegetative height).

Services	Function	Process	FD or vegetation Criteria	FD Criteria used	Direction relationship	Confidence	Reference
Water cycle	Leaves effect on the rainfall	Interception	LAI	CWMH	+	-	P. Calanca, S Lavorel
		leaf evaporation	Foliar angle	% legumes	+	-	P. Calanca
		dripping	Foliar angle	% legumes	-	-	P. Calanca; L. Merbold
	soil water	Infiltration	Root size	% forbs	+	-	C. Roumet
		runoff		No direct effect of the vegetation	\	=	P. Calanca
		Soil surface water evaporation	LAI	CWMH	-	-	S Lavorel
		percolation		No direct effect of the vegetation	\		P. Calanca
		% of water available for the plant	Root deepness richness and regularity	CWM LNC	+	-	(Wright *et al*., 2004). Roumet
			Transpiration rate	CWMSLA	+	-	(Wright *et al*., 2004), experts' agreement).
	Water uptake	Transpiration	LAI	CWMH	-	-	S Lavorel

Table.1.8: Relationships between functional diversity criteria and the ecosystem process/ state linked spiders and orthoptera conservation. The confidence of each relationship was assessed based on the experts and literature surveys. (CWM community weighed mean value, FR functional richness, FD functional dispersion, LDMC leaf dry matter content and H: maximal vegetative height).

Services		Function	FD or vegetation Criteria	FD Criteria used	Direction Relationship	Confidence	Reference
Orthoptera Diversity	Orthoptera Diversity	Food diversity	Diversity of leaves characteristic/ height heterogeneity	Number of plant sp	+	-	(Marini *et al*., 2008; Marini *et al*., 2009; Essl and Dirnböck, 2012; Kati *et al*., 2012) T Walter (Gardiner and Hill, 2006)
			Temperature	Ellenberg Temperature	+	=	
		Habitat	Structural Heterogeneity	FDH	+	=	(Marini *et al*., 2008; Marini *et al*., 2009; Essl and Dirnböck, 2012; Kati *et al*., 2012)
		Food quantity	Biomass quantity	CWMH	+	+	(Hutchinson and King, 1980; Wallis De Vries *et al*., 2007; Lavorel *et al*., 2011; Fleurance *et al*., 2012)
	web spiders diversity	Habitat	Temperature	Ellenberg Temperature	+	=	(Gardiner and Hill, 2006; Marini *et al*., 2008),T Walter
			% of species with hard steams	% of apiaceous	+	-	(Morris, 2000; Dennis *et al*., 2001; Jeanneret *et al*., 2003b)
			Height of the vegetation	CWMH	+	=	(Gibson *et al*., 1992; Scohier and Dumont, 2012)
		habitat diversity	structural heterogeneity	FDH	+	=	Scohier et Dumont., 2012 Gibson et al., 1992
	web spiders diversity	Food quantity	Orthoptera abundance		+	+	P. Jeanneret
			% of species with hard steams	% of apiaceous	+	-	(Morris 2000, Dennis et al. 2001, Jeanneret et al. 2003)
			Height of the vegetation	CWMH	+	=	(Gibson *et al.* 1992, Scohier and Dumont 2012)
		habitat quality	structural heterogeneity	FDH	+	=	(Gibson e*t al.* 1992, Scohier and Dumont 2012)
soil spiders diversity		Habitat	Diversity of litter thickness	FRLDMC	+	-	(Dennis *et al*., 2001)
soil spiders abundance		Food quantity	Orthoptera abundance		+	+	P. Jeanneret
		Habitat	structural heterogeneity	FDH	+	=	(Dennis et al. 2001) ; P. Jeanneret

Table.1.9: Relationships between functional diversity criteria and the ecosystem process/ state linked pollinator conservation. The confidence of each relationship was assessed based on the experts and literature surveys. (CWM community weighed mean value, FR functional richness, FD functional dispersion, LDMC leaf dry matter content and H: maximal vegetative height). Butterflynbsp, Bumblebeesnbsp , Beenbsp Number of plant species visited by the butterfly, bumblebees and bee, %Bumblebee, %Bee and % Butterfly percentage of species with butterfly, bumblebees and bee pollination

Services	Function	Process	FD or vegetation Criteria	FD Criteria used	Direction relationship	Confidence	Reference
Butterflies diversity	Food diversity	Imago food		Flower duration	+	+	(Jeanneret *et al.*, 2003a; Jeanneret *et al.*, 2003b)
			Nectar diversity	Butterflynbsp	+	+	(Jeanneret *et al.*, 2003a; Jeanneret *et al.*, 2003b; Öckinger *et al.*, 2006; Sjödin *et al.*, 2008; Marini *et al.*, 2009)
			Height of the vegetation	CWMH	+	=	(Kruess and Tscharntke, 2002; Pöyry *et al.*, 2006; Wallis De Vries *et al.*, 2007)
	habitat		structural heterogeneity	FDH	+	=	(Wallis De Vries *et al.*, 2001; Pöyry *et al.*, 2006; Skórka *et al.*, 2007; Wallis De Vries *et al.*, 2007; Sjödin *et al.*, 2008; Marini *et al.*, 2009; Farruggia *et al.*, 2012)
Butterflies abundance			Number of flowers	% Butterflysp	+	+	(Bergman *et al.*, 2008; Dumont *et al.*, 2009; Farruggia *et al.*, 2012; Scohier *et al.*, 2013)
	Food quantity	Larvae food	Diversity of leaves characteristic	Number sp	+	=	(Marini *et al.*, 2009)
	habitat		Height of the vegetation	CWMH	+	=	(Hutchinson and King, 1980; Kruess and Tscharntke, 2002; Bergman *et al.*, 2008)
Bumblebees diversity			Diversity of food	Bumblebeesnbsp	+	=	(Carvell, 2002; Goulson *et al.*, 2005; Goulson *et al.*, 2007; Sjödin *et al.*, 2008)
	Food	Nesting sites	Height of the vegetation	CWMH	^	+	(Carvell, 2002; Sjödin *et al.*, 2008)
	habitat		Diversity of litter thickness	FRLDMC	+	-	(Carvell, 2002; Mokany *et al.*, 2008)
Bumblebees abundance				%Bumblebee	+	=	
			nectar & pollen quantity	Bumblebeessp legumes %	+	=	(Carvell, 2002; Goulson *et al.*, 2005; Sjödin *et al.*, 2008; Scohier *et al.*, 2013)
	Food	Flower access	Height of the vegetation	CWMH	^	+	(Carvell 2002, Sjödin et al. 2008)
		Nesting sites	litter thickness	CWMLDMC	+	-	(Carvell 2002, Mokany et al. 2008)
	Habitat		Height of the vegetation	CWMH	^	+	(Fussell and Corbet, 1992)
Domestic bee abundance				Flower duration	+	+	(Pywell *et al.*, 2006; Albrecht *et al.*, 2007; Sjödin *et al.*, 2008; Hudewenz *et al.*, 2012), Decourty
			Number of flowers	%Bee	+	+	(Sjödin *et al.*, 2008; Albrecht *et al.*, 2010; Ebeling *et al.*, 2011)
	Nectar & Pollen quantity			Pollen Quantity	+	+	(Westrich, 1990; Backhaus, 1993; Sjödin *et al.*, 2008)
			Flower quality	Nectar quantity	+	+	
	Pollen Diversity		Beenbsp	Beenbsp	+	+	(Roulston and Cane, 2000; Arnold *et al.*, 2009; Alaux *et al.*, 2010)
			Flower duration	Flower duration	+	+	

Table.1.10: Relationships between functional diversity criteria and the ecosystem process/ state linked earthworms' conservation. The confidence of each relationship was assessed based on the experts and literature surveys. (CWM community weighed mean value, LDMC leaf dry matter content, LNC leaf nitrogen content and H: maximal vegetative height).

Services	Function	FD or vegetation Criteria	FD Criteria used	Direction Relationship	Confidence	Reference
Endogenous Earthworms abundance		exudates roots quality	% Legumes	+	=	
	Soil Food	Roots biomass	% Grass	-	-	
anecic Earthworms abundance		exudates roots quality	% Legumes	+	=	
	Soil Food	Roots biomass	% Grass	-	-	(Eisenhauer *et al*., 2009; Birkhofer *et al*., 2011)
		litter quantity	CWMLDMC	-	=	
			CWMLNC	+	+	
	Surface food	Litter quality	% Legumes	+	+	(Mokany *et al*., 2008; Eisenhauer *et al*., 2009; Birkhofer *et al*., 2011)
Epigic Earthworms abundance	Habitat	Soil humidity	Humidity Ellenberg	^	=	(Birkhofer *et al*., 2011)
		litter quantity	CWMLDMC	-	=	
			CWMLNC	+	+	
	Surface food	Litter quality	% Legumes	+	+	(Mokany et al. 2008, Eisenhauer 2009, Birkhofer et al. 2011)

Table.1.11: Relationships between functional diversity criteria and the ecosystem process/ state linked forage services. The confidence of each relationship was assessed based on the experts and literature surveys. (CWM community weighed mean value, FD functional dispersion, LDMC leaf dry matter content, LNC leaf nitrogen content, SLA specific leaf area and H maximal vegetative height).

Services	FD Criteria used	Direction Relationship	Confidence	Reference
	CWMLNC	+	=	(Pontes *et al*., 2007)
	CWMLDMC	-	=	(Pontes et al. 2007)
Biomass Yield	%Grass	+	=	(Baumont *et al*., 2011)
dOM	% Grass	+	-	R Baumont
Congestion	Legumes/grass			R Baumont
Nitrogen content	CWMLNC	+	+	R Baumont
nutrient stability	% of dicot	+	-	R Baumont
biomass stability	FDSLA	+	=	(Duru *et al*., 2012b)

Chapter 2: Estimation of the errors in the calculation of functional diversity criteria from databases

Abstract Chapter 2

The objective of this chapter is to a) evaluate the effects of different sources of inaccuracies on the calculation of functional diversity from functional trait databases, and b) to evaluate the effect of using different sets of botanical surveys on the estimation of functional diversity.

First, we propose a framework to evaluate the inaccuracies caused by the intraspecific variability in functional trait values, when using functional trait database with one trait value per species. We propose three key variables to evaluate the suitability of a functional trait database: (1) the coefficient of variation of the indexes between the studied plant communities, (2) the pattern of intraspecific variability and (3) the amplitude of intraspecific variability.

Secondly, different imputation methods (4 single imputation and 1 multiple imputation methods) for filling in missing data in functional trait databases were tested at species and community levels. Some of the single imputation methods were based on ecological hypotheses found in literature. The results showed that functional diversity may be calculated using a database with up to 30% missing data, when an appropriate imputation method is used.

Thirdly, we evaluated the error caused by the deletion or absence of minor species on the calculation of functional diversity.

Finally, we assessed the effect of the sampling area on the plant functional diversity.

Some criteria, such as functional identity, were very accurate for all the different inaccuracies; others had many inaccuracies, such as the functional dispersion with the intraspecific variability or the functional evenness with the deletion of minor species.

I. Introduction

Some of the functional diversity criteria proposed in the first chapter (Table1.4) are based on the relative abundance of certain functional groups or botanical families, such as the relative abundance of legumes. Others are calculated from functional traits and the species abundance using functional diversity indexes.
In this section, we presented different limitations in the use of functional diversity indexes linked to the use of functional trait databases and different sets of botanical surveys. This section contains different studies which evaluate the inaccuracies in functional diversity calculation caused by these limitations, and proposes a threshold of confidence based on the selected criteria.

A. Different errors on the utilization of functional trait databases

Different inaccuracies in the calculation of functional diversity may occur when using functional traits extracted from databases.
Generally, only one value per trait per species is recorded in functional trait databases. For a species with several values, the mean of these values is generally used. However, functional traits may greatly vary within a single species (intraspecific variability), especially because of climatic variation. This intraspecific variability challenges the use of only one mean value per species (Messier *et al.*, 2010; Violle *et al.*, 2012).
Our first methodological work, presented in the chapter, was therefore to test the effect of intraspecific variability on the calculation of functional diversity and propose some tests to conclude on the utilization of functional trait databases with only one value per species (part II of the present chapter).
In functional trait databases, missing data is a common problem. This missing data affects the accuracy of the calculation of functional diversity. Furthermore, some indexes cannot be computed if data is missing.
The second study tested the interest of using ecological hypothesis to impute missing data in functional trait databases (part III). Some of these imputation methods are based on ecological hypotheses. We have estimated the level of inaccuracy caused by the use of the imputation at species level and at community level.
One advantage of using functional trait databases is to be able to reuse previous botanical surveys. In this study, we chose to regroup surveys from previous studies in order to obtain datasets with large ecological gradients. Some problems may occur with the use of botanical surveys from different sources. The following paragraph presents some of the inaccuracies that occurred by using several sources of botanical surveys, and how we assessed the impact of these inaccuracies on functional diversity calculation.

B. Utilization of different datasets of botanical survey

In botanical surveys, some species are occasionally unidentified. Other species may be simply be absent in the functional trait databases. Some species (like trees) are only recorded at juvenile stages. For these species, their functional traits are only measured on adult individuals and cannot be used in grasslands.
Therefore, not all species recorded in a survey can be used for the calculation of functional diversity indexes. However, these species may still represent an important part of the abundance of the community. **The third study of this**

chapter aims to evaluate how many species can be deleted or absent from a survey, without creating too many inaccuracies (part IV).
When using a variety of databases, the protocol for conducting each botanical survey may have been different from one survey to the next. The variation in sampling area can be especially important. The relationship between survey area and number of species has been assessed in previous studies. However, the link between survey area and functional diversity has never been studied to the best of our knowledge. **We therefore tested the effect of survey area on the calculation of functional diversity, in order to evaluate the inaccuracies caused by the differences in sampling protocol; afterwards, some potential corrections were proposed (part V)**.

II. A framework for the utilization of functional trait database with only one value per species regarding the intraspecific variability of functional trait

This part is based on an article in preparation.

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A. Abstract

Functional diversity can be related to ecosystem services and can explain the community's species assemblage. One way to measure functional diversity is to use functional trait databases which record measurements of functional traits value by species. However, the values of functional traits may vary within a single species. The aim of this paper is 1) to test the robustness of a large set of functional diversity indexes to intraspecific variability in different conditions 2) to propose a framework for the utilization of functional trait databases. We virtually created an intraspecific variability and compared the value of several functional diversity indexes before and after integrating intraspecific variability, for 6 types of communities, 9 types of functional indexes and 4 different patterns for intraspecific variability. For each case, we defined the maximum percentage of intraspecific variability that an index can withstand (robustness threshold). These thresholds were compared between indexes, type of communities and intraspecific variability patterns. For each comparison, we proposed three key variables to evaluate the suitability of a functional trait database: (1) the coefficient of variation of the indexes between communities, (2) the pattern of intraspecific variability and (3) the amplitude of intraspecific variability. It appeared that in certain situations, functional trait databases should not be used to measure functional diversity. More research would be needed to improve understanding of intraspecific variability and its effect of functional diversity calculations.

Keywords: Plant functional trait, functional diversity, LEDA database, virtual and real communities.

B. Introduction

Functional diversity, which is the set of values of a functional single trait (or multiple traits) of the individuals (or species) of a community, can give information on life strategies, on community assemblage rules and on expected responses of the communities to environmental changes(McGill *et al.*, 2006). It is also linked to ecosystem services (de Bello *et al.*, 2010; Lavorel *et al.*, 2011; Lavorel and Grigulis, 2012). Functional diversity is now used for the analysis of many different ecological communities: grasslands (Violle *et al.*, 2012), forests (Laliberté *et al.*, 2010), fish (Mouillot *et al.*, 2013), and birds (Bonthoux *et al.*, 2013)

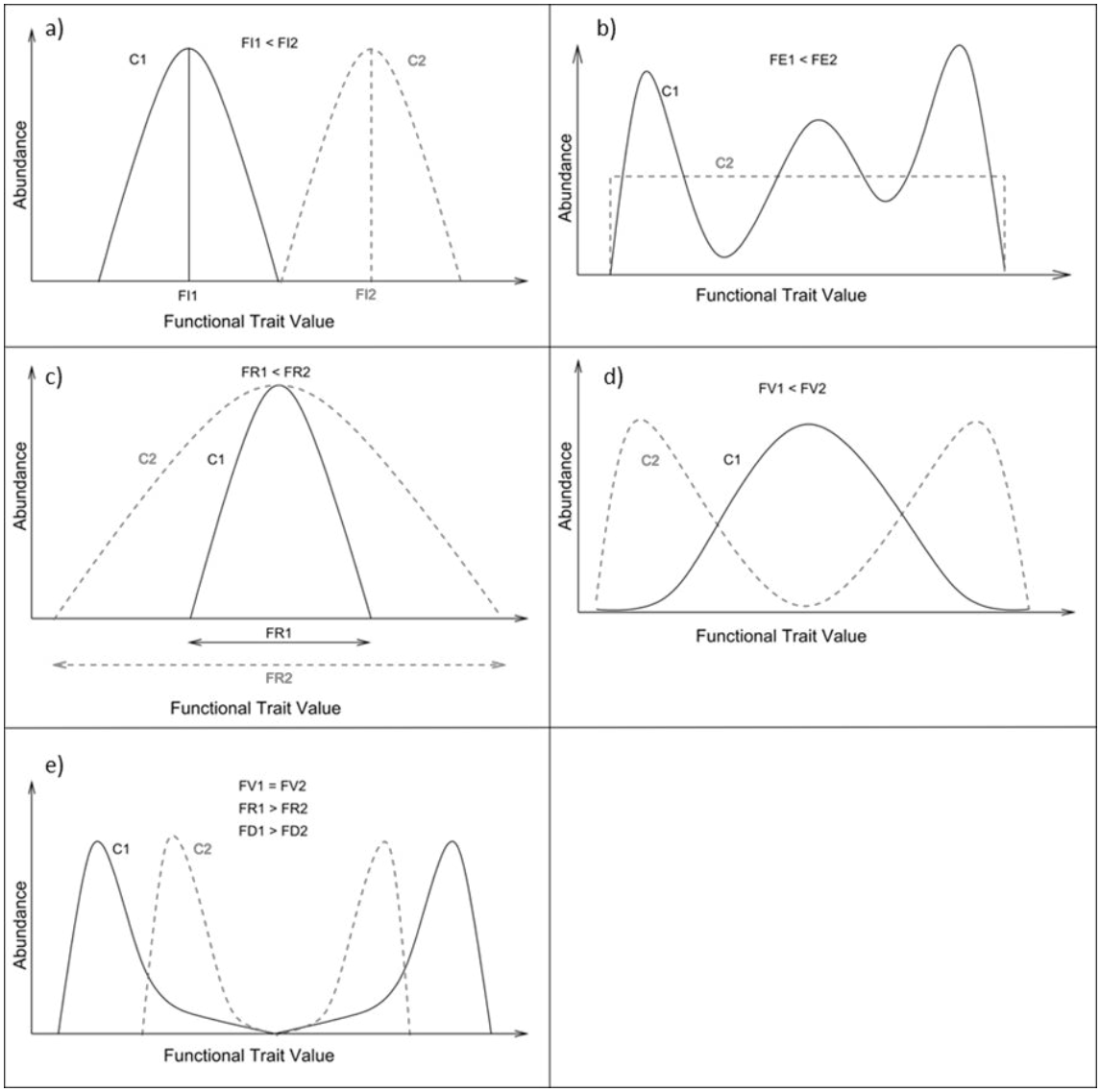

Figure2. 1: Schematic illustration of the different components of Functional Diversity used for the calculations. The x-axe represents the value of one functional trait; the y-axe represents the abundance of the species inside the community at the different values of the functional trait. In each case, two example communities (C1 and C2) with contrasted functional diversity situation are represented. a) Functional identity (FI), b) Functional evenness (FE), c) Functional richness (FR), d) Functional divergence (FV), e) Functional dispersion (FD).

Functional diversity is a complex notion that can be split in several components (Figure 2.1). Functional identity (FI) (Figure 2.1a) is defined as the community average value of a functional trait (Grime, 1998). Functional richness (FR) (Figure 2.1c) represents the amount of functional space occupied by a community of species or individuals (Petchey and Gaston, 2002; Mouchet *et al.*, 2010). Functional evenness (Figure 2.1b) (FE) corresponds to how evenly species' abundances are distributed in the functional space (Mouillot *et al.*, 2005; Mouchet *et al.*, 2010). Functional divergence (FV) (Figure 2.1d) defines how far high abundances species are from the center of the functional space (Mason *et al.*, 2005; Mouillot *et al.*, 2005). Evenness, divergence and richness are independent in virtual communities (Villeger *et al.*, 2008; Mouchet *et al.*, 2010) as well as in

grassland communities (Pakeman, 2011). So, a community with a very low functional richness can have a very high functional divergence and for some processes, both of these components are useful. So, Laliberté and Legendre (2010) propose the term of functional dispersion (FD) as a mix of functional richness and functional divergence (Figure 2.1e). A large set of indexes exist to assess these different components (Villeger *et al.*, 2008; Schleuter *et al.*, 2010). All the indexes use species composition of the community, their abundances and their functional traits.

Functional trait values can be measured *in natura* for the communities of interest but this is a very laborious undertaking in addition to floristic data acquisition. An alternative is to use values given in existing functional trait databases (FTD) (Kleyer *et al.*, 2008; Kattge *et al.*, 2011). A FTD contains measured values of functional trait for thousands of species. They are therefore powerful tools widely used in ecological research (Flynn *et al.*, 2009; Bernhardt-Romermann *et al.*, 2011; Pakeman, 2011; Thompson *et al.*, 2011). For the majority of species, only one value per trait is given. When several values per trait are given in the FTD, it is difficult to define which one fits best to one's own study (similarity/differences in pedo-climatic conditions, type of land use, geographical distance). Thus the mean value is often used instead (Cingolani *et al.*, 2007).

However, functional traits can vary considerably within a species, especially for plants (Garnier *et al.*, 2001; Roche *et al.*, 2004). Intraspecific variability (ITV) results either of genetic variability, either of the phenotypic plasticity of the species ("the capacity of a given genotype to render different phenotypic values for a given trait under different environmental conditions" (Valladares *et al.*, 2006)). This ITV can be high. Albert *et al.* (2010a) showed for 16 herbaceous plant species that the coefficient of variation for functional traits inside a species was between 0.19 and 0.49 for the vegetative height, between 0.08 and 0.25 for the leaf dry matter content (LDMC) and between 0.09 and 0.29 for the leaf nitrogen concentration (LNC).These values are consistent with several other studies (Garnier *et al.*, 2001; Roche *et al.*, 2004). Regarding these results, the use of only one functional trait value per species in functional ecology is questioned by recent papers (Albert *et al.*, 2010a; Albert *et al.*, 2010b; Messier *et al.*, 2010). The difference between the functional diversity indexes calculated with only the mean value and the indexes calculated with an integration of ITV can be significant (Albert *et al.*, 2011). The robustness of the ITV method depends on the indexes, the traits and the way variability is incorporated. The robustness can be defined as the maximum ITV that an index can support without altering the information.

One solution could be to develop new indexes able to include ITV in the calculation of functional diversity (Cianciaruso *et al.*, 2009; Schleuter *et al.*, 2010; de Bello *et al.*, 2011).These methods are only applicable with field measurements of the trait but they are unsuitable when the trait's values come from a FTD even with several values per species. Do researchers have to give up using FTD, or can FTD still be used in some cases? If the second hypothesis is true, a decision framework is needed to establish the case when a FTD can be used.

The aim of our study is to test the robustness of a large set of functional diversity indexes to ITV in various conditions (different types of communities, different pattern of ITV) and to propose, from these results, a framework to follow for identifying the case where functional diversity cannot be computed with a FTD with only one value per species. The robustness was evaluated from simulation

where different levels of ITV were virtually imputed. For each simulation, a comparison between the functional diversity indexes calculated with and without the ITV was made. The robustness threshold was defined as the point at which the levels of ITV are no longer considered acceptable.

C. Material &Methods

1. General procedure

A database containing the list of species and their abundance for 50 communities was crossed over with a FTD providing the mean value of four traits per species. Different types of samples and FTD were used (see paragraph 2). For each simulation, we used fifty samples randomly extracted from a larger pool of samples. We choose to use only fifty samples for two reasons: p values are not interesting for too many samples; and less that fifty samples would be too few for the analysis. For each simulation, the species / trait matrices of these different communities were used to calculate nine functional diversity indexes (see paragraph 4). An ITV for each trait of each species was then computed using four different methods (see paragraph 3). After computation of the ITV, a new set of species/trait matrices was obtained and the nine functional diversity indexes were re-calculated using these new matrices. The values of the functional diversity indexes before and after the inclusion of the intraspecific variation were then compared (see paragraph 5). The same protocol was repeated with different amplitudes (a) of ITV, increasing from 5 to 200% of the traits by step of 10%. This procedure was repeated one hundred times with a different set of communities for each amplitude of ITV

2. Relevés and functional trait databases used

Six different types of communities were used: three virtual communities and three grassland plant communities. The robustness differences between the six types of data were afterwards linked with the differences of the community structure (distribution of the index on the data); we tried to propose general rules for all type of communities.

a. Real relevés and trait values:

Three datasets of agriculturally managed grassland were used: one at the European scale, one at the regional scale and one at the local scale. For the three datasets, the four functional traits chosen for the simulations were the specific leaf area (SLA), the leaf dry matter content (LDMC), the vegetative height (H) and the seed mass (SM). The LEDA database (Kleyer *et al.*, 2008) was used to extract the four functional traits. The average trait values of the species were used when different values were proposed by the LEDA database. The four traits were normalized between 0 and 100. Unidentified species, species not documented in LEDA or species with missing data for the four traits were eliminated in the sampling. Samples were these eliminated species displayed a cumulated a total abundance higher than 5% were also eliminated. For the species with missing trait value in the FTD, we estimated the missing values using a method based on the dissimilarity between species. To replace the missing data of the trait T of the species S, the Gower dissimilarity(Gower, 1971) between S and the other species was calculated based on the other traits. For

species with Gower dissimilarity inferior to 0.05, the median of T was calculated and used to complete the missing data of the trait T of the species S.
For the local scale (Table2. 1 – CR1), the samples were provided by a time series of samples made on the INRA experimental farms (Gaujour 2012). 1556 surveys were available with 113 different species. For these 113 species, the vegetative height was missing for 5 species, leaf dry matter content for 32 species, the specific leaf area for 14 species and the seed mass for 7 species.
For the regional scale (Table 2.1 - CR2) we used only the Swiss dataset in the Alps (Peter *et al.*, 2008; Peter *et al.*, 2009). 593 surveys were available with 363 different species. For these 363 species, the vegetative height was missing for 16 species, leaf dry matter content for 90 species, the specific leaf area for 14 species and the seed mass for 15 species.
For the European scale (Table2. 1 - CR3), the samples were provided by three datasets: one in the Swiss Alps (Peter *et al.*, 2008; Peter *et al.*, 2009), one in eastern France (Plantureux and Thorion, 2005) and another one within a range of regions in France (Michaud *et al.*, 2012b). The three datasets add up 722 samples with 606 species. Imputation methods (gap filling) were used on only 20 species for H (3% of the data), on 136 species for LDMC (22%), 69 species for SM (11%) and 96 species for SLA (15%).

b. Virtual relevés and trait values:

Three virtual community's datasets (composition and abundance) were created using three different methods (random, broken stick and ecological gradients).
For the first virtual communities type (Table 2.1 - CV1), species compositions/abundances and the trait's values were randomly created. First, 150 virtual species were virtually created and for each species, the trait's values were randomly attributed using a uniform law between 0 and 100. The number of species was randomly selected between 10 to 50 species. From the pool of 150 species, species were randomly selected with a number equivalent to the previous number of species selected. The abundance of the species was also randomly determined. For each species, an "abundance" value between 0 and 100 was randomly created using a uniform law. The values of each species were divided by the sum of all the values of species inside a community. This way, a relative abundance of the species was randomly defined. 5 000 communities were created using this method (50 communities by simulation * 100 simulations).
For the second type of virtual community, a broken stick model (Mac Arthur, 1957) (Table2.1 - CV2) was used to compute the species composition and abundance. In the broken sticks model, the resources available for the plants are limited. Each species consumes part of the resources and its abundance was calculated as p*r, where p is the percentage of resources consumed and r is the initial amount of available resources. First, one species was randomly selected within a pool of 100 virtual species. This species used p_{sp1} % of r, so the abundance of the first species was p_{sp1}*r and r- p_{sp1}*r was still available for other species. p_{sp1} was randomly defined between 0 and 0.2 to 0.8. The upper limit for p_{sp1} was different for each community and randomly chosen between 0.2 and 0.8 in order to create a gradient of specific richness. The specific richness of our virtual communities was therefore not fixed. Another species was then randomly selected within the 99 remaining species. This species consumed p_{sp2} of the available resources (r- p_{sp1}*r) and its abundance was p_{sp2}*(r- p_{sp1}*r). The value of

p_{sp2} was determined by the same way as the value of p_{sp1}. Further species were sampled until the amount of resource available was inferior to 0.01*r. We created 50 virtual communities using this method. The virtual FTD contained four traits for each of the 100 virtual species; the trait data was obtained randomly using a uniform law between 0 and 100 for each trait of each species. 100 replicates of virtual FTD and of the set of 50 communities were created using this procedure.
For the third type of virtual community (Table 2.1 - CV3), the species compositions/abundances and the trait values were created using "ecological gradients". We assumed that the functional structure of the community was driven by four different environmental gradients as proposed by Keddy (1992). These gradients were defined between 0 and 100. For each ecological gradient we attributed a random value in each community, representing the position of the community on the four gradients. For each species, we defined a sensitivity to the gradient. This sensibility was defined by the two limits of the species on the gradient (lower and upper borders). The borders were the limit of the ecological niche of the species, for that ecological gradient. The borders were randomly attributed using a uniform law between 0 and 100. The first trait was defined from the first ecological gradient. The trait value was randomly attributed by a uniform law between the two borders of the species. The two limits of the uniform law were defined between the two borders of the species on the ecological gradient. The second, third and the fourth trait were defined from the second, the third and the fourth ecological gradient respectively. A pool of 1500 species was created using this approach. The species could not be present on a site with an ecological value outside these borders. These species were eliminated by ecological filters. In the end, only the species not eliminated after applying the 4 ecological filters, were present in the community. The species abundances were calculated from their borders on the 4 ecological gradients. A species was considered more abundant if it was close to the middle of its ecological niche. Conversely, a species would have lower abundance at the extreme end of its ecological niche. So, for each of the 4 gradients, we calculated the distance between the center of the ecological niche of the species and the position of the community on the ecological gradient, divided by the distance between the two values of the border of the ecological niche. These calculations were made for the 4 gradients. The minimal value of this calculation was used to define the abundance of the species. The relative abundance was calculated by dividing the abundance of the species by the sum of the abundance of all the species in the community. 5000 communities were created using this method (50 communities per simulation* 100 simulations).
The numbers of species in the functional trait databases were different between the 3 types of simulation. The objective was to obtain the same range of species richness.

Table 2. 1: Types of communities used for the simulation (H: height, SM: seed mass, SLA: specific leaf area, LDMC: leaf dry matter content).

	Type of communities	**Species composition**	**Abundance of species**	**Trait value**
CR1	Real	Relevés from a local scale	Visual estimation of the abundance	H, SM, SLA, LDMC from LEDA
CR2	Real	Relevés from a regional scale	Visual estimation of the abundance	H, SM, SLA, LDMC from LEDA
CR3	Real	Relevés from an European scale	Visual estimation of the abundance	H, SM, SLA, LDMC from LEDA
CV1	Virtual	Randomly selected	Randomly attributed	Randomly defined
CV2	Virtual	Randomly selected	Broken stick model	Randomly defined
CV3	Virtual	Defined from the sensitivity of species to virtual ecological gradients	Defined from the difference between the gradients value of the community and the optimum of the species	Defined from the optimum of the species for a virtual ecological gradient

3. Patterns of intraspecific variation

Albert *et al*. (2011) showed that ITV does not follow the same pattern for all traits and these authors highlighted the difference of robustness between different patterns. In our study, we tested 4 different patterns of intraspecific variation.

In the mean method, the value given in the trait database (t_{FTD}) is supposed to be the average of the trait for each species (numerous values for these species in the FTD). Therefore, the effective value of the trait varied around the FTD value. For a maximum amplitude (a) of the variation (as a percentage of the FTD value); the trait value, after computation of the intraspecific variation (t_{ISV}), fell between t_{FTD} - ($0.5*a*t_{FTD}$) and t_{FTD}+ ($0.5*a*t_{FTD}$). This method assumed that the value in the FTD was the average value of several values

In the single-value method, the value in the FTD (t_{FTD}) was not considered to be the average value of the trait for the species. This method supposed that t_{FTD} was only a single measure per species. This value could be on any position in the range of values of the species. So for each species, a value representing the position of t_{FTD} on the range of the species and thereafter called C was selected randomly between 0 and 1. The trait value after computation of the intraspecific variation (t_{ISV}) was selected randomly between t_{FTD}-$a/2*C*t_{FTD}$ and t_{FTD}-$a/2*(1-C)*t_{FTD}$. The hypothesis behind this method was that only one value in FTD was recorded by species, and so the average value of the species *in natura* was not necessary. The FTD value was not the center of the intraspecific variability and the variation could be higher in one direction.

In the convergence method, the intraspecific variation was unilateral and in direction of the community-level weighted mean value of the trait (CWM). The trait database value was thus compared to the CWM. So if t_{FTD}<CWM, the intraspecific variation (t_{ISV}) was selected randomly (uniform law) between t_{FTD} and $t_{FTD}+a*t_{FTD}$ and if t_{FTD}> CWM, t_{ISV} was selected randomly between $t_{FTD}-a*t_{FTD}$ and t_{FTD}. This method assumed that the functional trait of the individual converged in direction of the trait community level (aggregated trait, CWM). It was also

assumed that the trait at the individual level was driven by the same factors as the trait of species at community level.
In the divergence method, the intraspecific variation (t_{ISV}) was unilateral and in the opposite direction of the community-level weighted means of trait value (CWM). In this method, t_{FTD} was compared to CWM and if t_{FTD}<CWM, t_{ISV} was to be selected randomly between t_{FTD}-a*t_{FTD} and t_{FTD} using a uniform law. This method supposed that the functional trait of the individual diverged on the opposite direction of the community trait level. By the effect of competition, individuals could not have the same trait, leading to a divergence (Violle *et al.*, 2012). Some ecological filters could also select different values of functional trait, creating divergence.

4. Indexes of functional diversity

We chose different types of indexes for each component of functional diversity, except for the functional identity: a one-dimensional index and a multidimensional index. These univariate or multivariate indexes are presented on Table 2.2.
One-dimensional indexes, i.e. univariate indexes were computed for each trait and multidimensional indexes, i.e. multivariate indexes on the four traits together.

Table 2. 2: Functional diversity indexes used and their abbreviations.

Component	Univariate index (u)	Multivariate index (m)
Functional identity (FI)	FI:Community Weighted Mean	/
Functional richness (FR)	FRu :Range of the trait	FRm:FRic index (Villeger *et al.* 2008)
Functional evenness (FE)	FEu:FROm index (Mouillot *et al.* 2005)	FEm :FEve index (Villeger *et al.* 2008)
Functional divergence (FV)	FVu:FDs index (Schleuter *et al.* 2010)	FVm: FDiv index (Villeger *et al.*, 2008)
Functional dispersion (FD)	FDu:FDis index (Laliberté & Legendre 2010)	FDm: FDis index (Laliberté & Legendre 2010)

5. Evaluation of robustness after and before computation of intraspecific variability

We used a Pearson correlation test between functional indexes calculated before and after computation of intraspecific variation for the 50 communities of one simulation. For the 100 simulations, we noted the percentage of simulation where the p-value was superior to 0.05. We linearly interpolated these percentages in order to find the amplitude corresponding to a 95 percentage of simulation. We used this percentage as the robustness threshold (RT).
Afterward, we compared the different RT between the different indexes, patterns and types of data. These comparisons were made using a Kruskal-Wallis test followed by a multiple comparison test (Siegel, 1956). We also linked the RT of the different types of community to the distribution of the functional diversity indexes for each one. The coefficient of variation, the standard deviation and the difference between the minimal value and the maximal value divided by the median were calculated on the set of the different samples (5000 for the virtual communities and 722 for the CR3, 593 for the CR2 and 153 for CR1) used for the simulation, in order to describe the distribution of each functional index between the different types of data.

From all the results, we then proposed a framework to evaluate the influence of intraspecific variability on the use of functional trait databases.

D. Results

1. Presentation of one example of simulation

Figure 2.2 presents the results of one example of simulation for the plant grassland community at European scale (CR3 community) under a convergence pattern. For the vegetative height (H, Figure 2.2a), the most robust index was the functional evenness for an intraspecific variability amplitude of up to 200%. The second most robust index was the functional identity with a RT of 187%. The next most robust index was the functional richness (RT=175%), followed by the functional dispersion (RT=98.7%). The less robust of the functional indexes for H was the functional divergence (RT=40.3%).
The robustness of the indexes for leaf dry matter content (LDMC, Figure 2.2b) was lower than for H, considering the functional identity (RT=81%), functional richness (RT=70%), functional dispersion (RT=24.6%) and functional divergence (RT=36.2%).
For the seed mass, only the functional divergence with a RT of 145% and the functional identity (RT=154%) were affected by intraspecific variability between 1 and 200% of ITV (Figure 2.2c).
The specific leaf area (SLA, Figure 2.2d) was the trait most affected by the ITV in this simulation. As with the other traits, the functional evenness was never affected by ITV. Functional identity of SLA had a RT of 98.3%. Functional richness of SLA had a RT of 56.9%. The dispersion and the divergence for other traits had the lowest robustness, at 31.7% and 21.2% respectively.
For the multivariate index (Figure 2.2e), the hierarchy between functional diversity components was the same than with the univariated index. Functional evenness and functional richness computed from the four traits were always robust. The multivariate divergence index was less robust than the univariated indexes (RT=26.7%). The dispersion multivariate index had a RT of 68.3%.

2. Difference between indexes

Average RT of each index for the different ITV patterns are presented in Table 2. 3; those for the different types of community are presented in Table 2.4. Functional richness multivariate index was the most robust index (RT=192.6%). On all the cases (pattern*type of communities), except for two cases for the CV3 type of communities, the FRm index was never affected by ITV. Functional identity was the second most robust index with an average of 174.4%. Thus no difference was found between indexes for FRu (112.8%), FEm (127.6%), FVu (117.6%), FDu (116.7%), FDm (108.7%) and FVu (73.5%). Functional evenness with univariated index (67.0%) displayed the lowest robustness compared to the other indexes. However, the robustness of functional evenness was not different than the robustness of the FVm index.

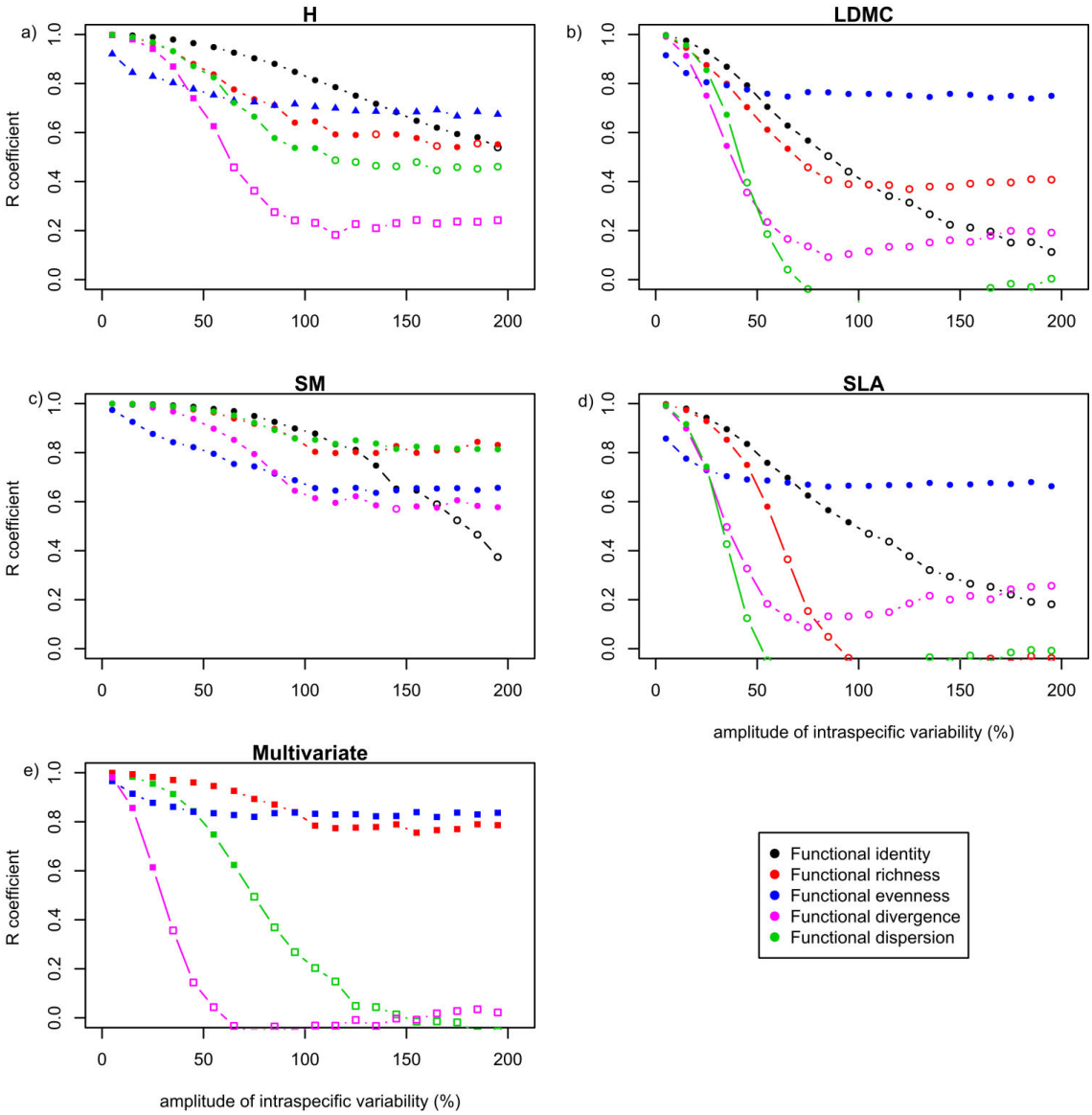

Figure2. 2: Effect of intraspecific variability on the R Pearson coefficient between functional diversity indexes before and after computation of intraspecific variability on the plant grassland community (CR3 type) with a convergence pattern. a) univariate index computed with vegetative height (H), b) univariate index computed with leaf dry matter content (LDMC), c) univariate index computed with seed mass (SM), d) univariate index computed with specific leaf area (SLA), e) multivariate index computed with all four traits. Each point represents the average R coefficient of 100 simulations (100 set of 50 communities). Full dots represent the level of ITV where at least 95% of the correlations were significant (P.value<0.05). Empty dots represent the simulation where less than 95% of the correlations were significant (P.value<0.05). Functional identity (FI) is drawn in black, functional richness (FR) in red, functional evenness (FE) in blue, functional divergence (FV) in purple and functional dispersion (FD) in green.

3. Differences between intra-specific patterns

The effect of the pattern on RT was very significant (Kruskal-Wallis chi-squared = 64.9; p-value = $5.181e^{-14)}$. Convergence pattern induced a lower RT (average of 86.4%) than the other patterns (Table 2.3). The robustness of the single value pattern (average of 112.6%) was higher than the robustness of the convergence pattern, but lower than the

mean and the divergence pattern. The divergence and the mean pattern had no difference with an average of 142.1% and 134.9% respectively.
Functional divergence indexes were the type of index most influenced by the convergence pattern. The average RT for the functional divergence multivariate index was lower by 80% for the convergence pattern, compared to the other patterns. However, the hierarchy between the indexes was not affected by the pattern (Spearman test).

4. Differences between types of communities

From a general point of view, the robustness was higher on the real communities (CR1 to CR3) that on the virtual ones (CV1 to CV3, Table2.4). Among the real communities, the type of community inducing the most important robustness was the CR3 (European scale) with an average RT of 156.7 %, followed by the CR2 type (Regional scale) with a RT of 137.04 % and the CR1 type (local scale) with an average RT of 123.3% (Table 2.4). For the virtual communities, the three types of community had nearly the same robustness with an average RT of 91.9 for V1, 105.1 for V2 and 100.0 for V3.
Some indexes were statistically influenced by the type of community used: FEm, FVu, FEu and FRu. For example, FEu was higher with R3 (200%) than with other type of communities. The FEu of CV1 (RT=15.4) and CV2 (RT=14.4) were lower than with other type of communities. FRu robustness was higher on all the real communities. Other indexes showed no difference between the different types of communities.

5. Relationships between the robustness and the distribution of indexes

We tested linear models of the coefficient variation against the robustness and the coefficient of variation against the original values (Table 2.5). The coefficient of variances of the indexes appeared as an explicative variable of the robustness of 5 indexes. For the simulations under the convergence pattern, the robustness of FEu indexes was linked the CV of the FEu between the different trait and type of communities (R^2=0.78***). The same relationship was also found for the FRu index (R^2=0.49**), the FVm index (R^2=0.87***), the FVu index (R^2=0.26*) and for the FDu index (R^2=0.78**). For the FDm index, only a slight tendency was observed (R^2=0.48 ns).
The difference between the minimum and the maximum, divided by the median, was also a good indicator of the robustness for the FRu (R^2=0.33**), the FEu (R^2=0.19*), the FVu (R^2=0.38**) and for the FDu (R^2=0.85***); it was not significant for FEm (R^2=0.46 ns).

Table 2. 3: Average robustness threshold of each index per pattern of ITV. Each value represents the lowest amplitude of intraspecific variation where less than 95 correlations of the 100 simulations were significant**.** When the indexes are still robust with 200%, the robustness threshold was defined as 200.

Index/pattern	Convergence	Divergence	Mean	Single-value	Average per index
Univariate indexes					
Functional identity (FI)	131.9	197.0	192.9	175.5	**174.4**
Functional richness (FRu)	95.1	120.5	135.6	100.1	**112.8**
Functional evenness (FEu)	64.1	68.0	72.5	63.3	**67.0**
Functional divergence (FVu)	57.5	178.1	130.0	104.6	**117.6**
Functional dispersion(FDu)	69.9	150.4	135.0	111.7	**116.8**
Multivariate indexes					
Functional richness (FRm)	176.2	194.2	200.0	200.0	**192.6**
Functional evenness (FEm)	117.1	137.3	130.3	125.7	**127.6**
Functional divergence (FVm)	34.9	113.6	87.5	58.2	**73.5**
Functional dispersion (FDm)	71.3	109.5	155.7	98.4	**108.7**
Average per pattern	**86.4**	**142.1**	**134.9**	**112.6**	**119.0**

Table 2. 4: Average robustness threshold of each index per type of community. Each value represents the lowest amplitude of intraspecific variation where less than 95 correlations of the 100 simulations were significant**.** When the indexes are still robust with 200%, the robustness threshold was defined as 200.

community/pattern	R1	R2	R3	V1	V2	V3	Average per index
Univariate indexes							
Functional Identity (FI)	159.7	167.2	168.6	182.0	180.2	188.5	**174.4**
Functional Richness (Uni) FRu	144.4	150.1	166.7	85.9	70.5	59.4	**112.8**
Functional Evenness (Uni) FEu	55.7	80.3	200.0	15.4	14.4	36.3	**67.0**
Functional Divergence (Uni) FVu	133.4	139.9	131.3	65.3	126.6	108.9	**117.6**
Functional Dispersion(Uni) FDu	136.8	138.2	130.9	92.7	118.2	83.8	**116.8**
Multivariate indexes							
Functional Richness (Multi) FRm	200.0	200.0	200.0	200.0	200.0	155.5	**192.6**
Functional Evenness (Multi) Fem	54.5	191.3	200.0	65.2	54.7	200.0	**127.6**
Functional Divergence (Multi) FVm	68.3	71.5	63.6	77.3	92.2	68.3	**73.5**
Functional Dispersion (Multi) FDm	116.6	123.6	107.4	98.1	137.0	69.6	**108.7**
Average per community	**123.3**	**137.0**	**156.7**	**91.9**	**105.1**	**100.0**	**119.0**

Table 2. 5. Average coefficient variation for robustness threshold of each index per pattern of ITV. Each value represents the lowest amplitude of intraspecific variation where less than 95 correlations of the 100 simulations were significant**.** When the indexes are still robust with 200%, the robustness threshold was defined as 200.

community/pattern	R1	R2	R3	V1	V2	V3
Univariate indexes						
Functional Identity FI	33.6	51.7	40.9	12.7	26.0	28.5
Functional Richness (Uni) FRu	53.8	42.1	72.3	6.8	7.1	9.7
Functional Evenness (Uni) FEu	38.4	23.1	48.7	13.3	26.8	16.2
Functional Divergence (Uni) FVu	78.7	103.0	87.9	22.9	56.4	66.4
Functional Dispersion(Uni) FDu	64.0	65.8	62.9	13.4	30.8	31.7
Multivariate indexes						
Functional Richness (Multi) FRm	238.7	116.8	245.1	44.8	49.5	29.9
Functional Evenness (Multi) Fem	28.0	10.5	45.3	11.3	22.9	12.2
Functional Divergence (Multi) FVm	19.5	17.4	16.7	4.0	11.6	13.5
Functional Dispersion (Multi) FDm	25.5	20.3	23.5	5.9	13.0	12.0

E. Discussion

1. Differences between Functional Diversity indexes

The functional identity (FI) index and the functional richness multivariate (FRm) index were the most robust index for ITV. These indexes were not affected by the type of community. Furthermore, they were calculated using a selected and "restricted" number of species. The community weighted mean value (FI index) depended only on the higher abundant species. Only the variation of the functional trait of the highest abundant species can affect the calculation of the functional identity index. The low effect of the intraspecific variability on functional identity was already shown on field measurements (Lavorel *et al.*, 2008) and by simulation (Albert *et al.*, 2011).
FRm value depends only on extreme species. Only a high ITV effect on these extreme species would affect the index value. We used the FRic index based on a convex volume (Cornwell *et al.*, 2006; Villeger *et al.*, 2008) for the calculation of FRm. Other indexes able to compute multivariate function richness depend on a higher number of species. For example, The FRD index is based on the functional distance between the species (Petchey and Gaston, 2002; Cornwell *et al.*, 2006; Mouchet *et al.*, 2008; Villeger *et al.*, 2008) and the FRis index also considers the functional gap between species (Schleuter *et al.*, 2010). These indexes depend on more species that the other indexes of functional richness. So we can suppose that the robustness of these indexes could be lower. However, the different indexes of functional richness, FRu, are related (Mouchet *et al.*, 2010; Schleuter *et al.*, 2010) and have almost the same behavior regarding the effect of ITV according to the studies of Albert *et al.*,(2011).
The unvaried functional richness (trait functional range) also depends on few species but it was less robust than FRm (Table 2.3). For FRm, one decrease in the range of one trait can be balanced by the increase of another trait. The very high coefficient of variation of FRm showed the large set of values taken into account by these indexes. It also explained the high robustness of these indexes.
The other indexes were less robust and were more affected by the distribution of the value of FEu was the less robust index. It could be related to the fact that the calculation of FEu index depended on all the species in the communities.

2. Difference between the types of communities

The type of data affects strongly the robustness of the indexes. FRu and FE indexes are more robust on real communities (Table 2.3). These differences of robustness between the types of communities can be explained by some characteristics of data. Indeed, the coefficients of variation of the different indexes are positively linked with the robustness (Table 2.3 and 2.4). It's mainly due to the fact that we defined the robustness from the modification of the ranking between communities. A same variation will have more effect on the ranking between communities in the case where all the communities have the same value (low coefficient of variations) than when the communities are widely distributed. A little difference induced by ITV has more impact on the classification among grassland for functional indexes. If the values of indexes are contained in a small range of value, the indexes will be more sensible to ITV.
The variability of the index on the studies sites is a key parameter to conclude on the utilization of a functional trait database with only one value per species

(Figure 2.3A). This is the first step of framework. The users of functional trait database have to evaluate the distribution of the studied indexes on the studied datasets. However, in order to evaluate these distributions, the data have to be recorded. It does not help to plan an experiment. Some information on the distribution of the functional diversity variables can be extrapolated from the literature. Indeed, the difference of the distribution of the indexes can be related with the community rules assemblages (McGill *et al.*, 2006; Mouchet *et al.*, 2010). The functional richness depends on ecological filters. Without ecological filters, species with very different values can cohabit inside the same community creating high functional amplitude for all the communities. It's the reason why the robustness is low for FRu in virtual communities especially for the CV1 and CV2 where no ecological filters influenced the species assemblages. On real communities, FRu is always lower and with more distributed values. Ecological filters like climate or disturbance induce a gradient of functional richness (Flynn *et al.*, 2009; Pakeman, 2011).
Another example to illustrate this effect of species assemblage is that FEu has a lower coefficient variation in the different virtual communities than on the real communities (especially in comparison with the European scale type of communities). For example in the CV2 communities, almost all the FE indexes values were between 0.3 and 0.4. In real communities, the values of FE indexes are more uniformly distributed with more extreme values. The presence of low evenness in real communities can be explained by the presence of functional optimums for a community. Indeed, all the abundance is regrouped around one or few trait values. The presence of high evenness in real community can be explained by the limiting similarity hypothesis (Stubbs and Wilson, 2004). It's assumed that species/individuals with same functional traits are not able to coexist in the same community by the effect of competition. Under this hypothesis, the abundances are more uniformly distributed between trait values. Depending on environmental conditions and on studied traits, the most structuring of the two hypotheses seems different (Bernard-Verdier *et al.*, 2012).
In the decision rules of Albert *et al.*, (2012), this question of the distribution of the data was indirectly addressed by the importance of the studied scale. The underlying idea was that at a large geographic scale, environmental factors will create a large gradient of trait values and so a large gradient of functional diversity. However we think that to consider only the scale of the study is not necessary a good indicator of the distribution of the functional diversity. The coefficient of variations of the different functional diversity indexes does not necessary increase with the augmentation of the study scale (Table 2.5). This absence of differences is due that in local conditions, some factors like levels of disturbance induce a strong selection of functional traits and so difference in functional diversity. More, most of functional diversity is found inside a community (de Bello *et al.*, 2009; Messier *et al.*, 2010). The spatial scale cannot be the only criteria to evaluate the potential variability of the functional diversity. In the studies where large gradient are studied, the errors induced by the ITV will be less important than studies where only one gradient is tested and the other gradients are controlled.

3. Importance of the intraspecific variability functional patterns

Patterns of ITV have a strong effect on the robustness of functional diversity indexes. The pattern did not affect the hierarchy between traits and only amplified

the difference. Albert *et al.* (2011) had already shown the importance of the pattern of intra-specific variation. Users of functional trait databases have to identify the pattern of the ITV of their studied traits under their studied conditions. The second step of our framework is the identification of the pattern of intraspecific variability. The best way for this identification is to use several trait values inside a species. The Ackerly diagram (Ackerly and Cornwell, 2007) is a good method for the identification of some ITV patterns using multiple trait value per species. The community weighted trait value (i.e. functional identity) is drawn in X axis and the species trait value on the Y axis. The line Y=X (the value of species trait is equal to the community trait value) is used as reference (Figure 2.3B). For the mean and single value pattern, the ITV is independent of the community value. So on the Ackerly diagram, the X value will be independent of the Y value. The main difference between these two patterns depended on the number of trait value per species in the functional trait database. The mean pattern is more likely when the trait value inside the database, can be considered as the mean trait value of the species. When only one or few value is recorded by species, the single value pattern seems more likely. The robustness is higher under the mean pattern that under the single value pattern. Indeed for the same amplitude, the variation of the trait could be more important in the single value pattern that in the mean pattern. In the literature, Albert *et al*., (2011) show that the intraspecific variability of LDMC and vegetative height were independent of community mean value. The value of the trait was optimal (maximal for vegetative height and minimal for leaf dry matter content) in the center of the ecological niche. The intraspecific variability follows a habitat suitability pattern.

Convergence and divergence patterns presume that the trait value of the species is linked with the community trait value. So; in the Ackerly diagram, the X value is linked to the Y value: positively for the convergence pattern and negatively for the divergence pattern. In our results, the convergence induces the worst robustness. However, it's hard to find an explanation to the very high robustness for the divergence pattern and the low robustness of the convergence pattern.

The last point of our framework is to quantity the intraspecific variability (Figure 2.3C). Indeed, high ITV will more challenge the use of functional trait database. The coefficient of variation of the trait inside a species is often proposed to measure ITV. It's mainly supposed that the distribution of the trait inside a species is normal. Another option is to quantify ITV by the amplitude of the trait (ri) (Ackerly and Cornwell, 2007). In order to compare traits, this amplitude is often divided by the average of the trait of the species (RI). This index is close to the index use on the study of plant plasticity (Valladares *et al*., 2006; Valladares *et al*., 2007). Some traits are less variable than other traits. For example on the LEDA trait database, for the grassland species we used on our simulations, the average ITV calculated with the Ri index of the vegetative height was of 105.9%, 30.1% for the LDMC, of 96.3 % for the seed mass and 65.2% for the SLA. These results are constituent with other results especially for the hierarchy between the traits (Garnier *et al*., 2001; Roche *et al*., 2004; Albert *et al*., 2010a; Albert *et al*., 2010b). This amplitude can be after compared to the robustness. The robustness threshold can be interpreted as the percentage limits of ITV that the indexes can support**.** If the relative amplitude (RI) of ITV is higher than the robustness threshold, we suppose that the indexes cannot be used with only one value per species in these conditions. We will develop in the following paragraph an

example based on the results of the robustness at European Scale (CR3) compared to the ITV measured on the LEDA database.

4. Can we use a functional trait database?

If we compared the amplitude of intraspecific variability recorded in the LEDA database and the robustness of the indexes on the grassland survey at European scale (CR3) with the convergence pattern (Figure2.2), seven indexes had a robustness threshold lower than the ITV of the LEDA database. For the SLA (ITV_{LEDA}=65.2%), it was the case for the functional divergence (RT=31.67%), dispersion (RT=21.5%) and the richness (RT =56.25%). For the vegetative height (ITV_{LEDA}=105.9%), the functional divergence (RT=40.29%) and dispersion (RT=98.75%) have a lower robustness than the amplitude of intraspecific variability of this trait in the LEDA. For the LDMC (ITV_{LEDA}=30.1%), only the functional dispersion is problematic (RT=24.63%). For Seed mass (ITV_{LEDA}= 96.3 %), all the index had a robustness higher than the amplitude of intraspecific variability. The functional divergence multivariate index (RT=26.66%) and dispersion (RT=68.33%) are less robust than the average ITV of the 4 traits (ITV_{LEDA}= 74.55 %). We choose the convergence pattern for this comparison. The convergence pattern was the worse of the different pattern we tested. The indexes more robust than the amplitude of ITV for the convergence pattern will be also robust for the other pattern. This pattern seems also to be the one for the SLA (Ackerly and Cornwell, 2007). For the LDMC and vegetative height, the pattern of ITV was different (Habitat suitability) (Albert *et al.*, 2011). We did not test this pattern but may be compared to the no mean pattern. In this case, all the indexes, calculated with LDMC and H, are more robust than the ITV measured on LEDA.

These comparisons show important limits of the use of functional trait database with only one value for studying some functional diversity indexes like the divergence and the dispersion. These components are more and more used on literature (Botta-Dukat, 2005; Cadotte *et al.*, 2009; de Bello *et al.*, 2009; Ricotta and Szeidl, 2009). Divergence and dispersion indexes indicate the repartition of the abundance inside the community and evaluate the number of different functional strategy. Divergence and dispersion give also information on the niche complementarily. These indexes are crucial to understand the ecological dynamics of the community. Dispersion (Rao) and divergence of some plant functional traits like the SLA can also be related with ecosystem functions (Mason *et al.*, 2005; Mokany *et al.*, 2008; Klumpp and Soussana, 2009).

For these variables, do we have to stop using FTD? And always make field measurement? In the more recent functional trait database, several measures of one trait are recorded for the same species with sometime information on the measured site like localization and environmental condition (Kleyer *et al.*, 2008; Kattge *et al.*, 2011). A partial solution could be to choose one value rather than the mean of these values. The important question is how to choose one of these values. The best way is to choose the value with the same environmental conditions. It's unlikely that for one trait, the measure was available for all the possibilities. Are the climate factors more important that soil factors or than agricultural management? This choice may be different between traits and species. More studies are also needed to test the effect of the environment on the intraspecific variability.

5. Conclusions

Our results show that intraspecific variability can have a strong influence on the calculation of functional diversity. We proposed a framework in three steps to evaluate the errors induced by the utilization of functional trait database with only one trait value per species. First, study the distribution of the indexes values on the selection of studied sites. More the values of the indexes will be wildly distributed less the intraspecific variability will impact the functional diversity studies. Secondly, understand the pattern of intraspecific variability is a key to estimate the robustness to intraspecific variability. When the trait values of species follow the same pattern as the value at community level, the robustness is lower. Logically, the more a functional trait is variable within a species the more difficult the use of functional trait database will be hard. The framework proposed in this article can be used to evaluate the errors induced by the ITV.

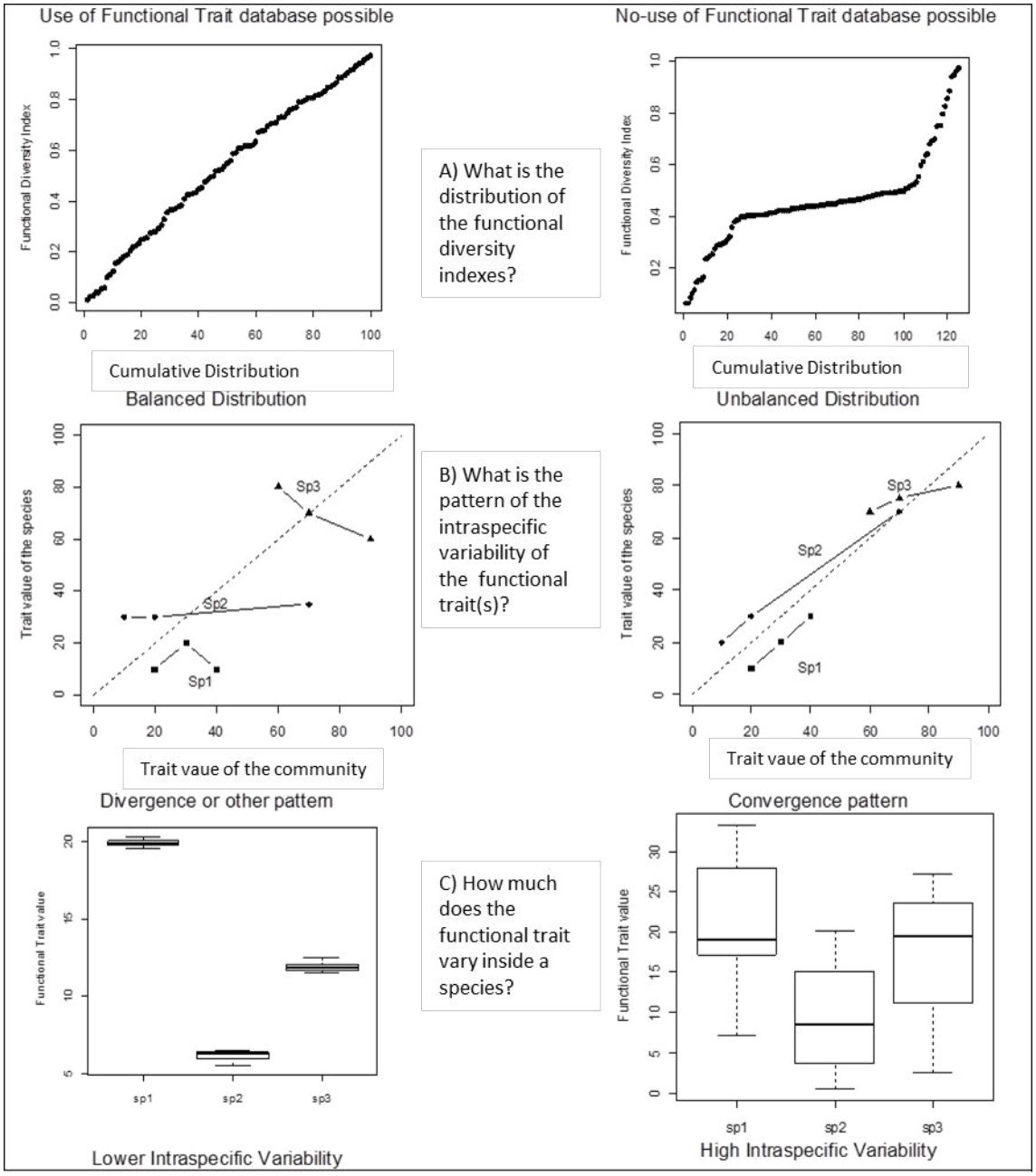

Figure2. 3: The three points to check before the use of a functional trait database: A) the distribution of the functional diversity indexes between communities, B) the pattern of intraspecific variability, C) the amplitude of intraspecific variability.

III. Filling the gap in traits databases Use of ecological hypotheses to replace missing data

This part is based on an article accepted in Ecology and Evolution in January 2014.

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A. Summary

1. Functional trait databases are powerful tools in ecology, though most of them contain large amounts of missing values.
2. The goal of this study was to test the effect of imputation methods on the evaluation of trait values at species level and on the subsequent calculation of functional diversity indices at community level using functional trait databases. Two simple imputation methods (Average and Median), two methods based on ecological hypotheses and one multiple imputation method were tested using a large plant trait database, together with the influence of the percentage of missing data and differences between functional traits. At community level, the complete-case approach and three functional diversity indices calculated from grassland plant communities were included.
3. At the species level, one of the methods based on ecological hypothesis was for all traits more accurate than imputation with average or median values, but the multiple imputation method was superior for most of the traits. The method based on functional proximity between species was the best method for traits with an unbalanced distribution, while the method based on the existence of relationships between traits was the best for traits with a balanced distribution.
The ranking of the grassland communities for their functional diversity indices was not robust with the complete-case approach, even for low percentages of missing data. With the imputation methods based on ecological hypotheses, functional diversity indices could be computed with a maximum of 30% of missing data, without affecting the ranking between grassland communities. The multiple imputation method performed well, but not better than single imputation based on ecological hypothesis and adapted to the distribution of the trait values for the functional identity and range of the communities.
4. Ecological studies using functional trait databases have to deal with missing data using imputation methods corresponding to their specific needs and making the most out of the information available in the databases. Within this framework, this study indicate the possibilities and limits of single imputation methods based on ecological hypothesis and conclude that they could be useful when studying the ranking of communities for their functional diversity indices.
Key-words: Functional diversity, missing data, LEDA database, plant functional trait, imputation methods

B. Introduction

Advances in ecological research, combined with the increasing power of statistical analyses and computers, allow researchers to study more and more species under an increasingly wide range of environmental conditions (Spiegelberger *et al.*, 2012). Ecological studies on plant community assemblages usually rely on large amounts of data compiled in databases, linking community assemblages and environmental conditions data with data about the functional traits of the species. Such databases are crucial for improving our understanding of the effects of global changes, like the loss of biodiversity or climate change, on the biosphere (Kattge *et al.*, 2011). This because on one hand, important plant functional traits are driven by environmental conditions (de Bello *et al.*, 2005b; Louault *et al.*, 2005; Ackerly and Cornwell, 2007; Ordoñez *et al.*, 2009), and on the other hand plant functional traits influence ecosystem functions, such as primary productivity and nutrient cycling (Mokany *et al.*, 2008; Klumpp and Soussana, 2009; de Bello *et al.*, 2010).

Standardized protocols are available for the measurements of plant traits in the field (Cornelissen *et al.*, 2003; Pérez-Harguindeguy *et al.*, 2013), and these measurements are now collected in large, well-structured databases (Kleyer *et al.*, 2008; Kattge *et al.*, 2011) accessible to the scientific community. However, plant trait databases contain a lot of missing data and probably will continue to for a long time because of the labor-intensive nature of collecting well-informed, standardized data, and because studies with different aims are usually interested in different traits. It is therefore unrealistic to expect complete knowledge of a large number of species from various ecosystems. For instance, in the large database of the TRY initiative (Kattge *et al.*, 2011), 39.1% of trait values concerned only four traits (specific leaf area, vegetative height, leaf dry matter content and seed mass, 13.2%, 10.0%, 8.7% and 7.2%, respectively). These four traits are frequently the best documented, and even for them, the percentage of missing data is high. For instance, in the LEDA database (Kleyer *et al.*, 2008); status in 2011) among the 8,195 registered species, only 2,019 species have information on specific leaf area (75% missing), 1,730 on leaf dry matter content (78% missing), 2,492 on seed mass (69% missing) and 2,893 for vegetative height (64% missing). Species with missing data are not generally the most dominant species observed in floristic relevés. Nevertheless, these missing data limit the optimal use of plant trait databases, as functional diversity indices, for instance, need to be calculated without missing values (Mason *et al.*, 2005; Villeger *et al.*, 2008).

An option still used to deal with this missing data is to delete species with missing data for the calculation of diversity indices (Lin *et al.*, 2011) .The obvious drawback is that it may introduce bias in the range of species retained for calculation and considerably reduce the dataset, consequently limiting the statistical power of any forthcoming analysis. Garnier *et al.*,(2004) suggested that this deletion is acceptable for estimation of the community weighted mean trait value (CWM) as long as it only concerns the minor species. They indicated that the deletion of minor species should not exceed 20% of the total biomass of the community. Indeed, if the value of a plant trait does not vary widely between species of a community, the weighted mean trait value of the community can be calculated with species that make up 80% of the total biomass of the communities. The additional effort required to sample species traits would not be worthwhile in terms of exactness (Pakeman and Quested, 2007). However,

exploring the effects of environmental constraints on plant community structure or the role of functional diversity in ecosystem processes without taking minor species into consideration could be misleading (Walker *et al.*, 1999), as minor species can have a significant effect on ecosystem function (Boeken and Schachak, 2006).

Another option used in some studies is to replace the missing data using an imputation method. In statistics, imputation is the process of replacing missing data with substituted values (Nakagawa and Freckleton, 2008). Imputation can be simple : missing data can be replaced by the mean or the median of the available trait values, as implemented in existing studies (Gunton *et al.*, 2011; Fried *et al.*, 2012). However, such simple imputation methods do not take the functional differences between species into account.

A third option, that is only relevant for functional diversity indices calculated from several traits, is to use the Gower distance and project the distance with a Principal Coordinate Analysis (Villeger *et al.*, 2008; Mouillot *et al.*, 2011). The Gower distance can be computed with some missing data (Gower, 1971) and the PCoA allows projection of a distance matrix on several axes, the axes being then used as functional traits. This method assesses the functional spaces but the traits information gets lost and only multivariate approaches can be used.

The problem of missing values in large matrices exists in a wide range of fields and advanced mathematical methods of imputation to deal with it have been developed, like multiple imputation (Schafer and Graham, 2002; Van Buuren *et al.*, 2006; Van Buuren, 2007; Azur *et al.*, 2011). Multiple imputation is a Monte Carlo technique in which the missing values are replaced by $m>1$ imputed values. Each of the imputed complete datasets is analyzed by standard methods, and the results are combined to produce estimates and confidence intervals that incorporate missing-data uncertainty (Nakagawa and Freckleton, 2008). We did not find any utilization of multiple imputation on functional trait databases. For the utilization of more advanced missing data imputation on functional trait databases, we only found the study of (Shan *et al.*, 2012) that recently tested another type of method: the hierarchical probabilistic matrix factorization coupled with phylogenetic information to replace missing values in plant trait databases. Functional proximity between species (Westoby *et al.*, 2002; Diaz *et al.*, 2004) and relationships between traits (Wright *et al.*, 2004; Wright *et al.*, 2006) could also be used for imputation, making a comprehensive use of the information available in the trait database. An alternative method to deal with missing functional trait data without deleting species and taking functional relationships between species and/or traits into account, would therefore improve the use of functional trait databases.

The aim of this study was to test imputation methods that integrate knowledge of relationships between species, but uses simple mathematics to impute missing data in order to calculate functional diversity indices based on functional trait databases. First, we tested the effects of several imputation methods on the evaluation of the trait values at the species level, using different levels of missing data and a range of functional traits with varying distribution. In a second step, the effects of these methods on the calculation of functional diversity indices at the community level were assessed for grassland communities.

C. Materials and methodology of imputation of missing data

1. Selection of two sub-databases without missing data and insertion of missing data

Initially, only the average trait values of the species in the LEDA database (Kleyer *et al.*, 2008) were used. 1,054 herbaceous and ligneous plant species with no missing data for 9 continuous traits were found in the database and retained to establish the "whole sub-database" (Figure2.4 - step 1). These traits were vegetative height (H), reproductive height (RH), seed mass (SM), seed shape (SS), seed number per plant (SNP), specific leaf area (SLA), leaf dry matter content (LDMC), leaf mass (LM) and leaf surface (LS).

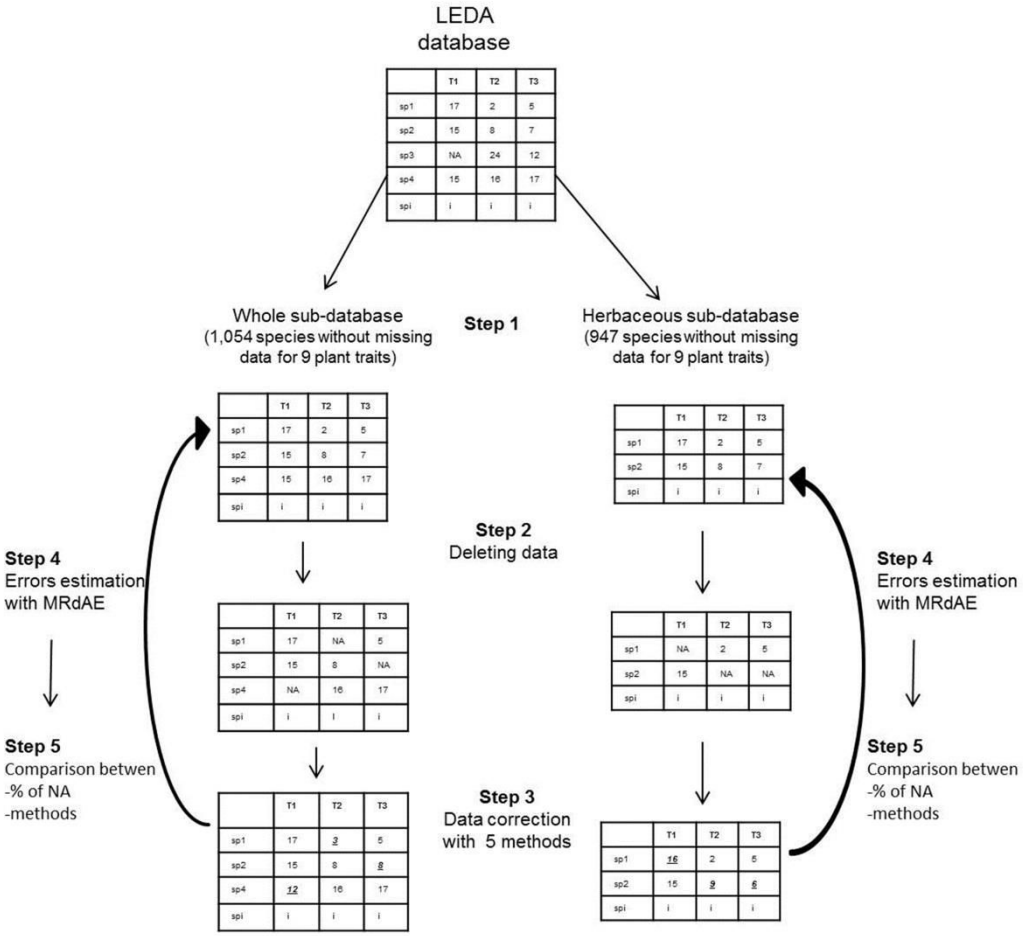

Figure2. 4: General procedure of estimation of errors for the imputation of missing data Step1) creation of two trait sub-databases, one with no species filters and one only with herbaceous species; Step2) missing data were inserted with 10 different percentages from 1% to 46%; the insertion was made 100 times per percentage of missing data (2000 different sub-databases were created this way); Step3) these missing data were replaced using 5 different methods (10000 corrected datasets were created this way); Step4) errors induced by the imputation were estimated by comparison between the original database and the corrected one; Step5) the error estimations were then compared between the different percentages of missing data for each method and between methods.

Within this sub-database, the distribution of the trait values were similar for the vegetative height (H), the reproductive height (RH), leaf mass (LM), leaf surface

(LS), seed shape (SS), seed mass (SM), seed number per plant (SNP). For these seven traits, most values were low with few extreme high values. The presence of a few tree species in the database is one reason for the unbalanced distribution of some traits. The distribution of the LDMC and the SLA values was close to a Gaussian distribution.
We also used a sub-database with only herbaceous species to assess how strongly the error induced by the imputation methods depended on the distribution of the trait values in the database (Figure2.4 - step 1). This second sub-database was set by eliminating the species with a vegetative height greater than 2 m and of the Raunkier types “phanerophyte” or “chamephyte” in order to eliminate trees and shrubs from the whole sub-database. In this second sub-database, called “herbaceous sub-database,” 947 species were documented with the same nine plant traits as for the whole sub-database. The vegetative height (H) and the reproductive height (RH) had a normal distribution in the herbaceous sub-database.
In these two sub-databases, missing data were deliberately inserted by randomly deleting existing values (Figure2.4 - step 2). Each existing value in the sub-database had a given probability of being deleted. Ten different probabilities of deletion were applied (from 0.01 to 0.46 with an interval of 0.05; same probability for all values of the sub-database at each step), yielding large differences in the level of missing data insertion. For each level of missing data, the random deletion was made 100 times. The deleted values could be different for each simulation. One-thousand different versions of the two sub-databases were created (10 levels of deletion probability x 100 random deletions).

2. Imputation methods

Five imputation methods were tested (Figure2.4 – step 3). These included two simple mathematical methods (“Average” and “Median”), as well as three methods that to our knowledge have not yet been implemented for imputation in functional trait databases: two methods based on ecological hypotheses and thereafter called the “Dissimilarity” and the “Relationships” methods, as well the multivariate imputation by chained equations (a multiple imputation method) (Azur *et al.*, 2011; Van Buuren and Groothuis-Oudshoorn, 2011).
The two single mathematical imputation methods consisted of either replacing the missing data with the average trait value (Average method) or by the median value of all species with documented values (Median method). These methods have already been used in literature (Gunton *et al.*, 2011; Fried *et al.*, 2012). In these two methods, the missing values of trait T_i for the species S_i to S_j are all replaced with the same value, without using the information that could be available from other traits.
The Dissimilarity imputation method is based on the functional proximity between species. This method relies on the hypothesis that species with the same functional strategy have a similar set of functional traits (Westoby *et al.*, 2002; Diaz *et al.*, 2004). To replace the missing data of the trait T_j of the species S_i, the Gower dissimilarity (Gower, 1971) between S_i and the other species, is calculated based on the other traits. The species showing high similarity with S_i are then selected, and the median of their trait values for T_j is computed and used to evaluate the missing value T_{ji}. We chose a Gower dissimilarity coefficient of 0.05 as threshold for species with high similarity. The Gower dissimilarity can

be computed with missing data, so the presence of other missing data would not disrupt replacement of the missing data.
The Relationship imputation method depends on the existence of relationships between plant traits (Wright *et al.*, 2004; Wright *et al.*, 2006). For each trait (T_j), the dataset is split in two matrices, according to the presence or absence of missing data for T_j: the first matrix containing all species with no missing data for T_j and the second matrix with all species with missing data for T_j. On the first matrix, a statistical model explaining T_j using the other traits is created by a stepwise regression. Then, this model is used to estimate the missing data in the second matrix. When, in a few cases, the value of another trait T_2 entering in the model for the estimation of the missing value T_{1i} was also missing for S_i, we replaced the missing value of T_{2i} with the median trait value of T_2. The occurrence of such a replacement of the missing value of another trait increased with increasing percentage of missing data.
R scripts (R, 2011) used to implement the two methods based on ecological hypothesis are available by request to the authors.
The four methods presented above perform simple imputation (each missing entry is replaced by a single imputed value). The resulting imputed dataset therefore contains genuine as well as simulated data. With such methods the uncertainty associated with imputed data is lost and cannot be propagated to the analyses to be applied on the imputed dataset. In contrast, the objective of the multiple imputation approach is to handle missing data in a way resulting in valid statistical inference, rather than to predict missing values as close as possible to the true ones (Rubin, 1996). Concretely, m different imputed values are generated for each missing entry, leading to m different imputed datasets. Analyses (here functional diversity indices computation) are then carried out on each imputed dataset and pooled to produce estimates and confidence intervals that incorporate missing-data uncertainty. We here also used a method of multiple imputation: the Multivariate Imputation by Chained Equations (MICE, Azur *et al.* 2011). The MICE method was computed using the “mice” package of R (Van Buuren and Groothuis-Oudshoorn, 2011). This method of imputation use predictive mean matching with five imputations. For the species level, the average of the 5 values imputed was used to replace the missing value.

3. Comparison of the errors between methods and percentage of missing data

For each simulation, we compared the original plant trait value $(To)_{ij}$ and the value after replacement $(Tr)_{ij}$ (Figure2.4 - step 4). The quality of the replacement was evaluated by an indicator independent of the number of missing data: a modified median relative absolute error (MRdAE) of the imputed values (MRdAE = median(abs $(To-Tr)_{ij}$) / median$(To)_{ij}$).The modification as compared to the MRdAE used in Hyndman & Koehler(2006) is that the dominator is equal to the median of the original value instead of abs(To(ij)-median(To(j)) Indeed, we wanted to assess the deviation from the original value of the functional trait rather than to compare two variables.
In our study, this indicator is more suitable than other common error measures such as the root mean square error for two major reasons. First, the MRdAE does not depend on the number of estimated values (i.e. the rate of missing values). Secondly, it is less sensitive to outliers (Hyndman and Koehler, 2006).

A Kruskal-Wallis nonparametric ANOVA test was realized on the MRdAE between the 10 different probabilities of missing data for each trait, for each method, and on the two sub-databases. This analysis was made 90 times (9 traits x 5 methods x 2 sub-databases). When the Kruskal-Wallis p value is not significant, it means that for a given sub-databases, the replacement method creates the same error during the replacement irrespective of the percentage of data that was missing and replaced. On the contrary, when the Kruskal-Wallis p value is significant, the accuracy of the method depends on the percentage of missing data (Figure2.4 - step 5).
We also compared the MRdAE between the four different methods using a Kruskal-Wallis nonparametric ANOVA. The comparison was made for each trait on each dataset but without separating the levels of missing data (analysis run 18 times: 9 traits x 2 datasets) (Figure2.4 - step 5). A multiple comparison test after Kruskal-Wallis (ad hoc test) was conducted (Siegel, 1956).

D. Results of imputation methods at the species level

1. Differences between the imputation methods on the whole sub-database

The Average method was the least accurate (higher MRdAE) for all the traits studied. The MRdAE of the Average method was highly variable between traits, from 0.25 for the SLA to 44.62 for the SNP (Table 2.6). The Median method was less accurate than the Dissimilarity method for all traits except for the SNP, but it was more accurate than the Relationships method except for the SLA and the LDMC. The Relationships method was therefore in most case less accurate than the Dissimilarity method. For the SLA and LDMC, the MRdAE of the five methods was low with similar values (around 0.24) (Table 2.6). For the other traits (H, RH, LM, LS, SS and SNP), the MRdAE of the single imputation methods was higher than for the SLA and the LDMC and ranged from 0.49 (RH with Dissimilarity method) to 45.00 (SNP with Average method). The differences between the methods were also more distinct with these traits than with the SLA and the LDMC (Table 2.6). The MICE method was more accurate than all other methods for all traits except for the specific leaf area.

2. Differences between methods on the herbaceous sub-database: effect of the trait distribution

The use of the herbaceous sub-database affected the results only for the vegetative height (H) and the reproductive height (RH) (Table 2.6). The distribution of these two traits was unbalanced for the whole sub-database and balanced for the herbaceous sub-database (results not shown). The MRdAE of the five imputation methods were lower for the herbaceous sub-database in comparison to the whole sub-database for these two traits. The minimal MRdAE of the single imputation methods was less when working with herbaceous plants only (and therefore with a balanced distribution of the traits) rather than with the whole sub-database (0.22 and 0.48, respectively; Table 2.6). Moreover, the Relationships method was more accurate than the Dissimilarity method for H and RH when using the herbaceous sub-database rather than the whole sub-database. No difference in accuracy ranking of the Relationships and the Dissimilarity methods was found between the whole sub-database and the herbaceous sub-database for the other traits because their distributions remain unchanged. In comparison with the whole sub-database, the accuracy of the

MICE methods for the H and the RH was higher with the herbaceous sub-database (MRdAE 0.21for H and MRdAE of 0.20 for RH).

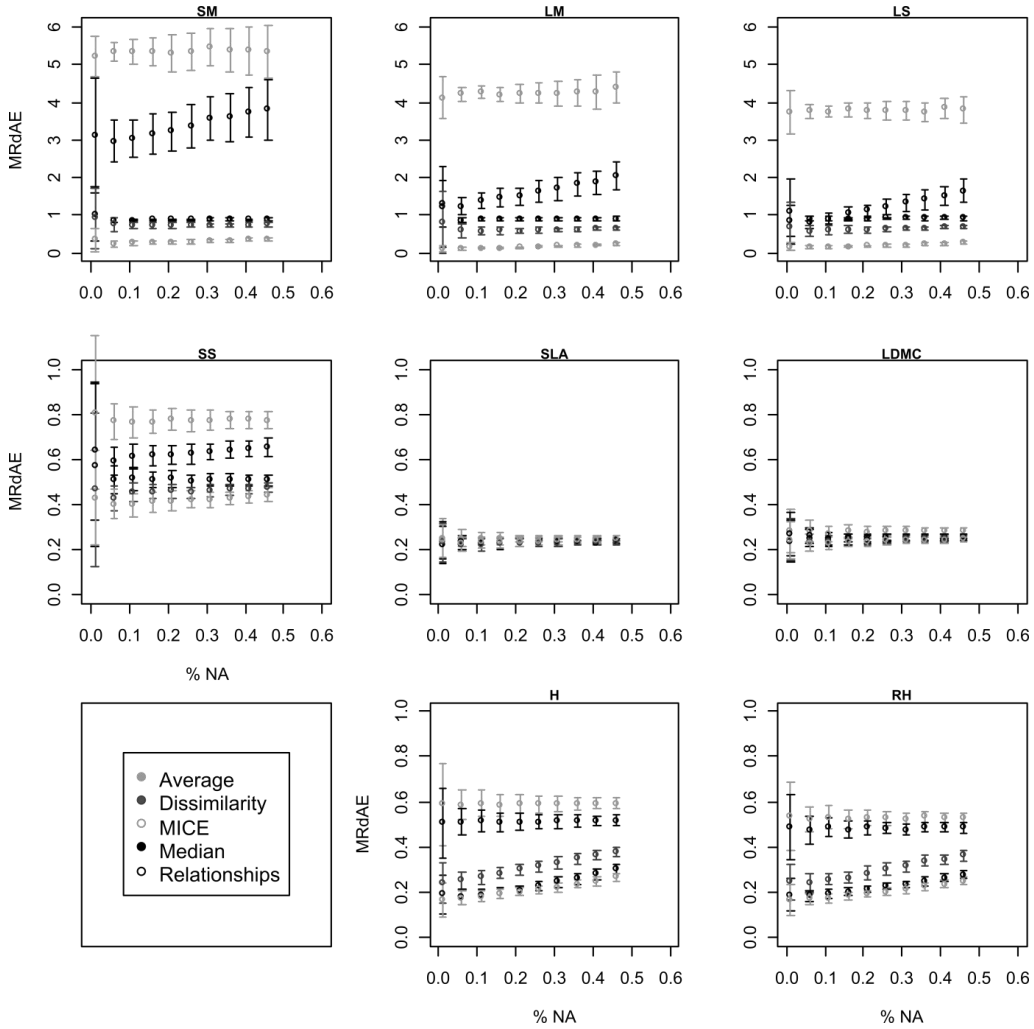

Figure2. 5: Evolution of the MRdAE of 5 imputation methods in the herbaceous sub-database with different percentages of missing data for 8 traits (SM: seed mass, LM: leaf mass, LS: leaf surface, SS: seed shape, SLA: specific leaf area, LDMC: leaf dry matter content, H: vegetative height, RH: reproductive height). The mean of 100 simulations ± the standard deviation is shown for each percentage of missing data.

3. Effect of the level of missing data

The Average method was not affected by the percentage of missing data on the two sub-databases except for the SNP with the herbaceous sub-database (Table 2.7). The Median method was only affected by the percentage of missing data for the SNP on the two sub-databases and the SM in the herbaceous sub-database). The Dissimilarity method was affected for seven traits in the herbaceous sub-database and only for four traits in the whole sub-database. The Relationships method was the most sensitive to the level of missing data. This method was affected by the percentage of missing data for five traits for the herbaceous sub-databases and eight traits for the whole sub-database (Table 2.7). The MICE method was affected by the percentage of missing data for six traits on the whole sub-database and 7 for the herbaceous sub-database.

Table 2.6: MRdAE(median relative absolute error) for each imputation method in the two sub-databases, averaged over all percentages of missing data (1 to 46%). A Kruskal-Wallis ANOVA and its ad hoc test were conducted to test the effect of the different methods on the MRdAE. A lower MRdAE means less error due to the imputation of missing values. The letters in a column correspond to the results of the ad hoc test. (H: vegetative height, LDMC: leaf dry matter content, LM: leaf mass, LS: leaf surface, RH: reproductive height, SM: seed mass, SNP: seeds number per plant, SS: seed shape, SLA: specific leaf area)

		H		LDMC		LM		LS		RH		SM		SNP		SS		SLA	
	Methods	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc
Whole database	Average	1.158	a	0.276	a	4.570	a	4.203	a	1.061	a	7.561	a	44.623	a	0.791	a	0.253	a
	Median	0.531	d	0.262	d	0.918	d	0.941	d	0.516	d	0.904	d	1.168	d	0.515	c	0.239	d
	MICE	0.253	b	0.235	b	0.164	b	0.192	b	0.259	b	0.242	b	0.283	b	0.426	b	0.241	b
	Dissimilarity	0.495	c	0.252	c	0.779	c	0.834	c	0.477	c	0.798	c	1.356	c	0.480	b	0.231	c
	Relationships	0.735	e	0.242	e	1.685	e	1.210	d	0.770	e	4.123	e	44.263	a	0.647	d	0.232	e
	Methods	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc	MRdAE	Adhoc
Herbaceous database	Average	0.590	a	0.281	a	4.241	a	3.774	a	0.528	a	5.342	a	38.180	a	0.776	a	0.250	a
	Median	0.512	d	0.258	d	0.926	d	0.932	d	0.483	d	0.893	d	1.158	d	0.516	d	0.233	cd
	MICE	0.209	b	0.239	b	0.159	b	0.194	b	0.201	b	0.303	b	0.257	b	0.421	b	0.238	b
	Dissimilarity	0.306	c	0.242	c	0.625	c	0.641	c	0.295	c	0.763	c	1.504	c	0.460	c	0.230	c
	Relationships	0.226	e	0.249	e	1.597	e	1.185	e	0.223	e	3.354	e	34.725	e	0.629	e	0.233	d

Table 2.7 Effect of percentage of missing data on the MRdAE (median relative absolute error) for the four methods applied to the two sub-databases. For each method, a one way Kruskal-wallis test was conducted to test the effect of the percentage of missing data on the MRdAE. The P-values are presented in the table for each method and each trait. (H: vegetative height, LDMC: leaf dry matter content, LM: leaf mass, LS: leaf surface, RH: reproductive height, SM: seed mass, SNP: seeds number per plant, SS: seed shape, SLA: specific leaf area).

		Traits								
	Methods	H	LDMC	LM	LS	RH	SM	SNP	SS	SLA
	Average	0.55	0.44	0.24	0.11	0.34	0.38	0.06	0.48	0.38
	Median	0.72	0.22	0.42	0.97	0.33	0.55	**0.01**	0.66	0.37
	MICE	0.22	**0.00**	**0.00**	**0.00**	0.11	**0.00**	0.53	**0.01**	**0.00**
	Dissimilarity	0.46	0.87	**0.00**	0.11	0.25	**0.00**	**0.01**	**0.01**	**0.00**
Whole sub-database	Relationships	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**	**0.00**	0.40	**0.00**	**0.00**
	Methods	H	LDMC	LM	LS	RH	SM	SNP	SS	SLA
	Average	0.69	0.55	0.07	0.07	0.47	0.32	**0.01**	0.80	0.95
	Median	0.89	0.03	0.00	0.19	0.20	**0.01**	**0.00**	0.46	0.89
	MICE	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.36	1.00	**0.01**	**0.00**
	Dissimilarity	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.52	**0.00**	**0.00**	0.07
Herbaceous sub-database	Relationships	**0.00**	0.31	**0.00**	**0.00**	**0.00**	**0.00**	0.36	0.39	0.16

E. Discussion of the accuracy of the imputation methods

The results show that at the species level the most accurate imputation method is not the same for all traits and in all cases, but one of the methods based on ecological hypothesis (Dissimilarity and Relationships methods) was always the most accurate among the single imputation methods. The relatively low MRdAE values found with at least one of the ecological methods for all the traits included in this study, particularly with the herbaceous sub-database, indicate the potential of these methods for the replacement of missing values prior to the calculation of functional diversity indices.

Among the single imputation methods, the Dissimilarity method is the most accurate when the trait distribution is unbalanced, as in leaf mass or leaf surface (Table 2.6). In this situation, the Median method is almost as accurate as the Dissimilarity method, whereas the Relationships method does not perform well on very unbalanced traits (like SNP) because the multilinear model is strongly governed by extreme values. However, when the trait distribution is more balanced, the accuracy of the Relationships method is similar (LDMC and SLA for the two sub-databases) or slightly better than that of the Dissimilarity method (H and RH for the herbaceous sub-database).

The multivariate imputation in chained equations was the most accurate method for the unbalanced trait (H, RH, SM, SNP, SS, LM and LS). For the SLA, the MICE method induces slightly more error that the ecological based methods. For the other balanced traits (LDMC and H or RH for herbaceous sub-database), the difference between MICE and the Relationships method was low. In the MICE method, the correction model can be adapted to the distribution of the variable (Azur *et al.*, 2011; Van Buuren and Groothuis-Oudshoorn, 2011), so that the traits with an exponential distribution are well corrected. This explains the higher accuracy of the MICE method on the unbalanced functional traits.

Comparing the results obtained with the two sub-databases, the error was lower when the traits had a balanced distribution (with the Relationships method) than when the traits had an unbalanced distribution. It seems better to choose a sub-database with balanced trait distribution, by for example, only using herbaceous species for grassland studies rather than all type of plants species. Traits' distributions explain the differences in accuracy observed between the single imputation methods, the traits, and the sub-databases. The key parameter to choose the adequate imputation method is thus the distribution of the value of the trait in the dataset. This also indicates that applying a transformation method to improve the distribution of the trait values prior to using an imputation method could be useful in improving the quality of the replacement.

The objectives and methods of the study should also be considered when choosing the imputation method. For instance, replacing the missing data using distances between species (Dissimilarity method) would not be an appropriate choice for a study on functional distance between species, as functional distance would then be underestimated. Functional distance between species is often used to classify species into groups or to calculate some functional diversity indices (Rao, 1982; Mouchet *et al.*, 2008).

The Relationships method is very sensitive to the percentage of missing data (Table 2.7). This could be due to the replacement of missing values of other traits by the median value of these traits that was needed for the creation and the utilization of the multi-linear models. The negative effect of these replacements on the accuracy of the estimated values increased with an increasing percentage of deleted data (Figure 2.5). The Dissimilarity method is less affected by the percentage of deleted data. Indeed the metric use to calculate the dissimilarity, the Gower dissimilarity coefficient is able to deal with missing data up to a certain threshold. Nevertheless, the Gower dissimilarity cannot be calculated between two species if no trait is documented for both species, and so the correction would not be possible if missing data are too numerous. In the hierarchical probabilistic matrix factorization method tested by Shan *et al.,* (2012),

phylogenetic information from an independent source is used to create groups of plants with trait values of reduced variability and the mean of the existing trait values is used to predict missing values within such groups. Shan *et a*l., (2012) showed that this method is satisfactory to predict trait values when information at the genus level is available. Instead of phylogenetic information from another database, the method considered here use relationships between traits, and hence all the information available within the trait database, and the mathematics involved are simpler. It is thus comparatively straightforward to apply. On the other hand, while the method propose by Shan *et al.*, (2012) needs only at least one trait value per plant, the method considered here requires several traits per plant/species to be documented.
In the different studies on missing data and imputation, the distribution of the missing data is a key parameter (Schafer and Graham, 2002; Nakagawa and Freckleton, 2008). Three different types of distribution of the missing data are described in the literature: missing completely at random (MCAR), missing at random (MAR) and missing not at random (MNAR). In functional traits databases, missing data will seldom be missing completely at random (MCAR), because the missingness is related to the frequency of the species and their abundances. Indeed, the probability that a species was measured and implemented in the functional trait database is higher if this species is frequent and/or abundant than if it is seldom. Nevertheless, we found no relationships between the functional trait values of the 9 traits and the frequency and average abundance of the species in our relevés dataset used for the calculation of functional diversity indices (below). Therefore, the missingness of the data in the original trait database was probably not related to the value of the traits. Regarding the trait values, the missingness produced by our random deletion was therefore similar to the missingness in the original database.
Our results present the error induced by different methods of imputation at the species level. Functional trait databases are often used to compute functional diversity indices of communities and it is therefore necessary to evaluate the effects of imputation of missing data at community level.

F. Effects of the imputation methods for the calculation of functional diversity indices

4. Material and method

We tested the effect of missing data and the difference between the methods of imputation on the computation of three functional diversity indices at the community level using grassland communities' data. These indices were the community weighted mean value of the trait (functional identity), its functional range, as well as its functional divergence. The functional range of the traits (difference between the minimum and the maximum) is important to understand the rules of plant community assemblage (Petchey and Gaston, 2002, 2006; Mouchet *et al.*, 2010). The functional divergence corresponds to the repartition of the abundance regarding functional identity within a plant community (Mason *et al.*, 2005; Mouchet *et al.*, 2010). We chose the functional divergence index proposed by Schleuter *et al.*,(2010) among the several indices available for the calculation of functional divergence.

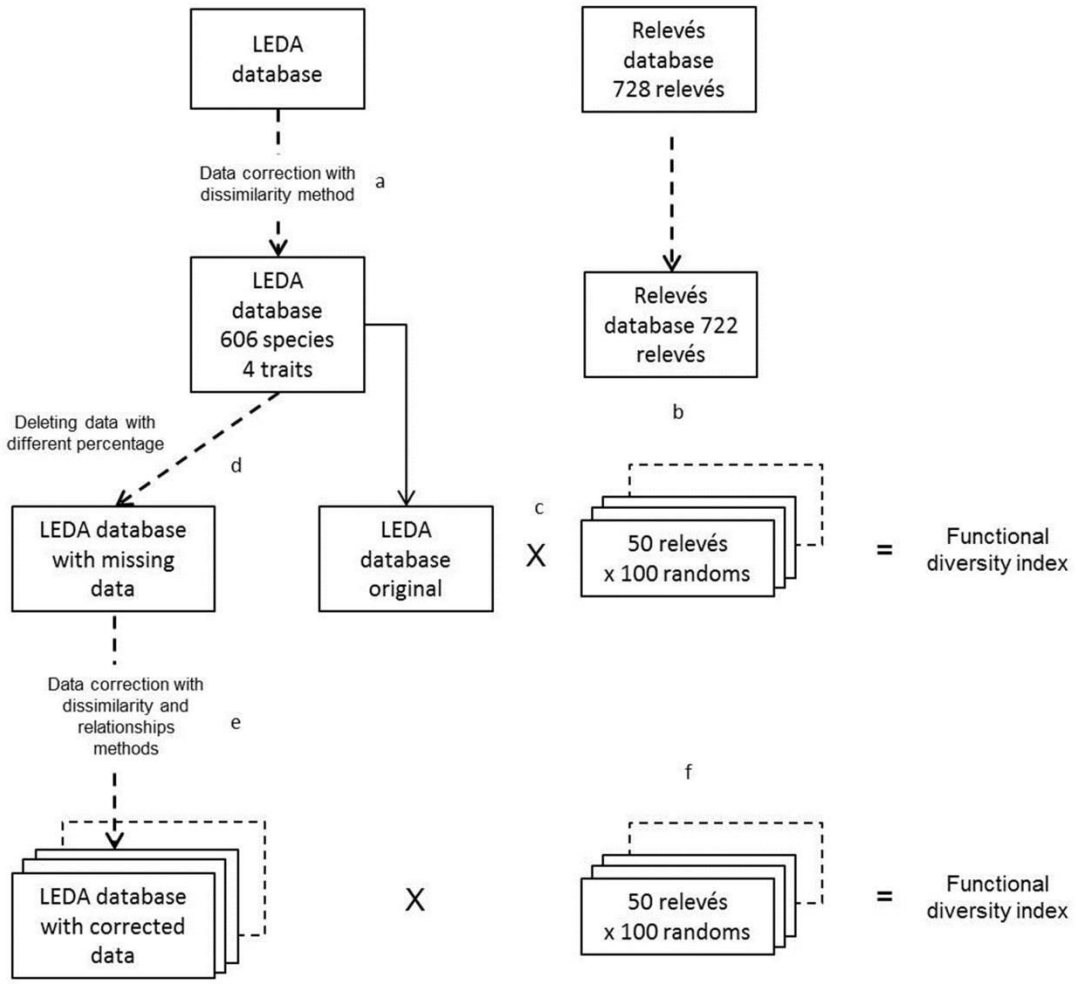

Figure2. 6: General procedure for the assessment of the effects of the imputation methods for the calculation of functional diversity indices. a) a database without missing data was created from the LEDA database (4 traits for 526 species; some replacement of missing values by the dissimilarity methods where necessary); b) 50 relevés were randomly selected from a large set of relevés (this process was repeated 100 times); c) 50 relevés and the trait database were crossed and functional diversity indices were computed; d) missing data were inserted in the trait database with several percentages; e) missing data were replaced with the Dissimilarity and the Relationships methods; e) these corrected databases were crossed with the 50 relevés, and functional diversity indices were computed; f) the indices computed from database without missing values were compared to the indices computed from the databases with replaced missing values using a Pearson correlation test.

The functional traits were extracted from the LEDA trait database (Kleyer *et al.*, 2008), Figure2.6). We limited the trait selection to 4 traits (SLA, SM, H, and LDMC) often used in grassland studies: The SLA, H, and SM are, for instance, the traits proposed on the leaf-height-seed (LHS) model of Westoby *et al.*,(2002), which is useful to assess the live strategy of the species. Moreover, LDMC and SLA are important traits in the leaf economic spectrum and are often linked with ecosystem function.

The grassland botanical relevés originated from 3 datasets: one from the Swiss Alps (Peter *et al.*, 2008; Peter *et al.*, 2009), one from the Vosges mountains in North-eastern France (Plantureux and Thorion, 2005) and another from a broader range of regions in France from Atlantic to continental conditions (Michaud *et al.*, 2012b). The grassland relevés used represent a large gradient of ecologic filters (climatic and agricultural management).

Our first attempt involved only relevés where all the species have a value for the four traits in the database. However, only 4 relevés fell within this constraint. Therefore, in order to start our test with enough data for the species present in the relevés, the

missing trait values in the LEDA database had to be imputed. Imputation was used on 20 species for H (3% of the data), on 136 species for LDMC (22%), 69 species for SM (11%) and 96 species for SLA (15%). The Dissimilarity method was used, as it proved satisfactory for the H, LDMC and the SLA in the first part of the study. SM, for which the Dissimilarity method was less accurate, had only 11% of missing values. Species unidentified in the surveys and species with missing data for the four traits were omitted. Only the relevés where the abundance of these unidentified species was inferior to 5% of the total abundance were kept. After these modifications, 722 relevés were available with 606 species.

The use of the dissimilarity imputation before the insertion of missing data induced some circularity in the evaluation of the imputation method. However, we think that the circularity is low. This circularity would be very problematic if a trait value was imputed twice the same way. In our work, this probability of double imputation is very low. Indeed, the imputation of one value depends of all the different trait values of the other species and also the missing data on the entire functional trait database. Indeed, the calculation of the dissimilarity would differ between two calculations if the missing data are not exactly on the same trait values. The selection of the close species in the dissimilarity method is related to the calculation of the Gower dissimilarity and so to the distribution of missing data in the functional trait database. Secondly, the calculation of the median of the trait value of the close species depends also on the presence of missing data for the functional trait value of these species.

Different other option could have to use: only use the dominant species in the survey (80% of the abundance) or virtually assemble species. The use of only dominant species would leave out the minor species. If we only interest of the dominant species, the percentage of missing data would be quite low and so the necessity of imputation would be less important.

The creation of artificial species assemblages with only species having a value for the 4 traits in the database would have yield unrealistic differences in functional diversity indices of the communities, because the majority of these species would have been common and thus ubiquist species. Thus, we consider that replacing some missing trait values in true communities to create a complete database as comparison point for our study was the most appropriate option.

Among these 722 relevés, for each simulation, we randomly selected 50 different relevés. This random selection was made 100 times in order to have 100 sets of 50 plant communities (Figure2.6b). Each set of relevés was crossed with the functional trait database.

We deliberately inserted missing data in the trait database, by randomly deleting some trait values (Figure 2.6d), and so created datasets with different percentages of missing data (1%, 5%, 10%, 20%, 30%, 40% and 50%). For each percentage the insertion of missing data was made 100 times (one insertion per set of 50 communities). These missing data were then replaced using the Dissimilarity, the Relationships or the MICE method (Figure2.6e) in order to create functional trait databases with imputed data. We did not examine imputation by the median or the average on the calculation of functional diversity indices, because at the species level one of the two ecological methods was always better or as good as the two mathematical methods (Table 2.7). The 50 communities were crossed with these trait databases with different percentages of replaced missing data and functional diversity indices were computed (Figure2.6 f). For the MICE method, the functional diversity indices were computed for each of the 5 imputations and the average value of these 5 estimations of the diversity indices was used for the comparison. The indices calculated from the values of the datasets with imputed values were compared to those calculated from the original database (without missing data) using a Pearson correlation test. From this comparison, we assessed the effect of replacing missing data on the ranking between the functional diversity indices of 50 grasslands. The p-value was calculated for each correlation between the two rankings for 100 sets of 50 grasslands. In most studies on functional diversity, the ranking between communities is more important than the absolute value of the

functional diversity. We thus focused on the effect of replacing missing data on this ranking. For the discussion we use the following threshold: if the correlation p-value was not significant for 5 or more of the 100 sets of communities, the results obtained by the imputation methods were considered unsuitable (by similitude with significant threshold at 5%). The percentage of missing data, for which this threshold was exceeded, was estimated by linear estimation between the simulations with the different levels of missing data.
We also conducted the simulation on the ranking of the communities for their functional diversity indices after deleting the species with a missing value (deletion option, also known as "complete-case analysis").
As discussed in the first part of this study, using the Dissimilarity method might underestimate the functional distance between the species. We could therefore suppose that this method could be problematic previous to calculation of the functional range of the communities. However, the imputation was computed on the functional trait database with the 606 species. Species with extreme trait values in a community might not be functionally isolated in the database, so that the imputed values are not necessarily forced toward the median of the community. The ranking of the communities for their functional range was similarly affected by the percentage of replaced data with the Dissimilarity as with the Relationships or the MICE methods.
Multivariate functional diversity indices like those propose by Villeger *et al.*(2008) were not tested. Thus, the replacement method proposed here cannot be compared with the method of the Gower dissimilarity follow by a PCoA. However, Gower dissimilarity can only be computed between two species with at least one common trait documented and the PCoA can only be implemented if all the pairwise distances between species are known. This method will therefore only be useful for a low percentage of missing data or/and a large number of traits. Results on the effect of imputation methods on functional diversity indices

a. Community weighted mean (functional identity)

When the missing data were replaced using the Dissimilarity method, the ranking between grasslands based on the community weighted mean (CWM) values was not affected by the percentage of missing data until more than 40% of the data were missing for SLA, LDMC and H. For the CWM of SM, the ranking was impacted by the imputation from 31% of missing data upwards (Table 2.8). The R Pearson coefficients were slightly higher for H and SM than for SLA and LDMC (Figure 2.7a1). When the missing data were replaced using the Relationships method, the ranking of grasslands based on the CWM was never affected by the percentage of missing data for H, SLA and LDMC. For SM however, this ranking was affected as soon as 15% of the data had to be imputed with the Relationships method (Figure 2.7 a2). When the missing data were replaced using the MICE method, only the ranking for SM was affected by the imputation (from 14% of missing data upwards; Table 2.8).

b. Functional range

With the imputation of missing data using the Dissimilarity method, the ranking between grasslands on the functional ranges of SLA and LDMC was never affected by the percentage of missing data. For SM and H, this ranking was affected by a percentage of 32%, respectively 40%, or more of missing data (Figure2.7b1). With the imputation of missing data using the Relationships method, the ranking between grasslands for the functional range of SLA and LDMC was never affected by the percentage of missing data. For H, the ranking was only significantly affected by missing data when 45% of data were missing, while for SM it was affected as soon as 12% of the data were missing (Figure2.7b2). Imputation with the MICE method led to different ranking between the grasslands compared to the ranking obtained with the

original database starting 39% of missing data for H and 17% for SM, while the ranking was not influenced by the percentage of missing data for SLA and LDMC (Table2.8).

c. Functional divergence

The R Pearson coefficient between functional divergence indices calculated without missing data and with data imputed with single imputation decreased faster with increasing percentage of missing data as for the functional identity of functional range indices (Figure2.7). With imputation using the Dissimilarity method, the ranking between grasslands for the functional divergence of SLA, LDMC, and SM were affected by the percentage of missing data from 31-40% of missing data upwards. The functional divergence of H was affected by the percentage of missing data starting 25% of data missing (Figure 2.7c1). With imputation using the Relationships method, the ranking between grasslands for the functional divergence of H, SLA and LDMC was affected by the percentage of missing data when 33-37% or more of the data were missing. The functional divergence of SM was affected by the imputation already starting 5% of missing data (Figure 2.7c2). With the MICE method, the ranking of the grasslands based on the divergence indices was not affected by the percentage of missing data (Table 2.8).

Table 2.8 :Percentage of missing data at which the p-value of the correlation between the ranking of the communities calculated without missing data and with imputed data became not significant for 5 of the 100 sets of communities, using the MICE, the Dissimilarity or the Relationships imputation methods, or the deletion of species with one missing trait value. (H: vegetative height, LDMC: leaf dry matter content, SM: seed mass, SLA: specific leaf area).

	Methods of imputation, resp. deletion			
	MICE	Dissimilarity	Relationships	Deletion
	Functional identity			
H	\	45	\	11
LDMC	\	43	\	6
SM	14	31	15	10
SLA	\	42	\	7
	Functional range			
H	39	40	45	14
LDMC	\	\	\	33
SM	17	32	12	7
SLA	\	\	\	23
	Functional divergence			
H	\	25	33	10
LDMC	\	31	37	8
SM	\	40	5	7
SLA	\	32	37	10

d. Deletion of species with missing trait values

The ranking between communities was quickly affected by the deletion of species with missing trait values for the four functional traits studied: 8% of missing data for CWM, 19% for functional range and 9% for the functional divergence in average over the four traits (Table 2.8).

5. Discussion of the effects of the imputation methods on functional diversity indices

The results clearly show the superiority of the tested imputation methods over the deletion of species with missing trait values for the estimation of functional diversity indices of grassland communities. They also show that single imputation methods that can be interpreted in ecological terms or Multivariate Imputation by Chained Equations can be used to replace missing data in a functional trait database in order to calculate functional diversity indices, with only few effects on the ranking between communities.

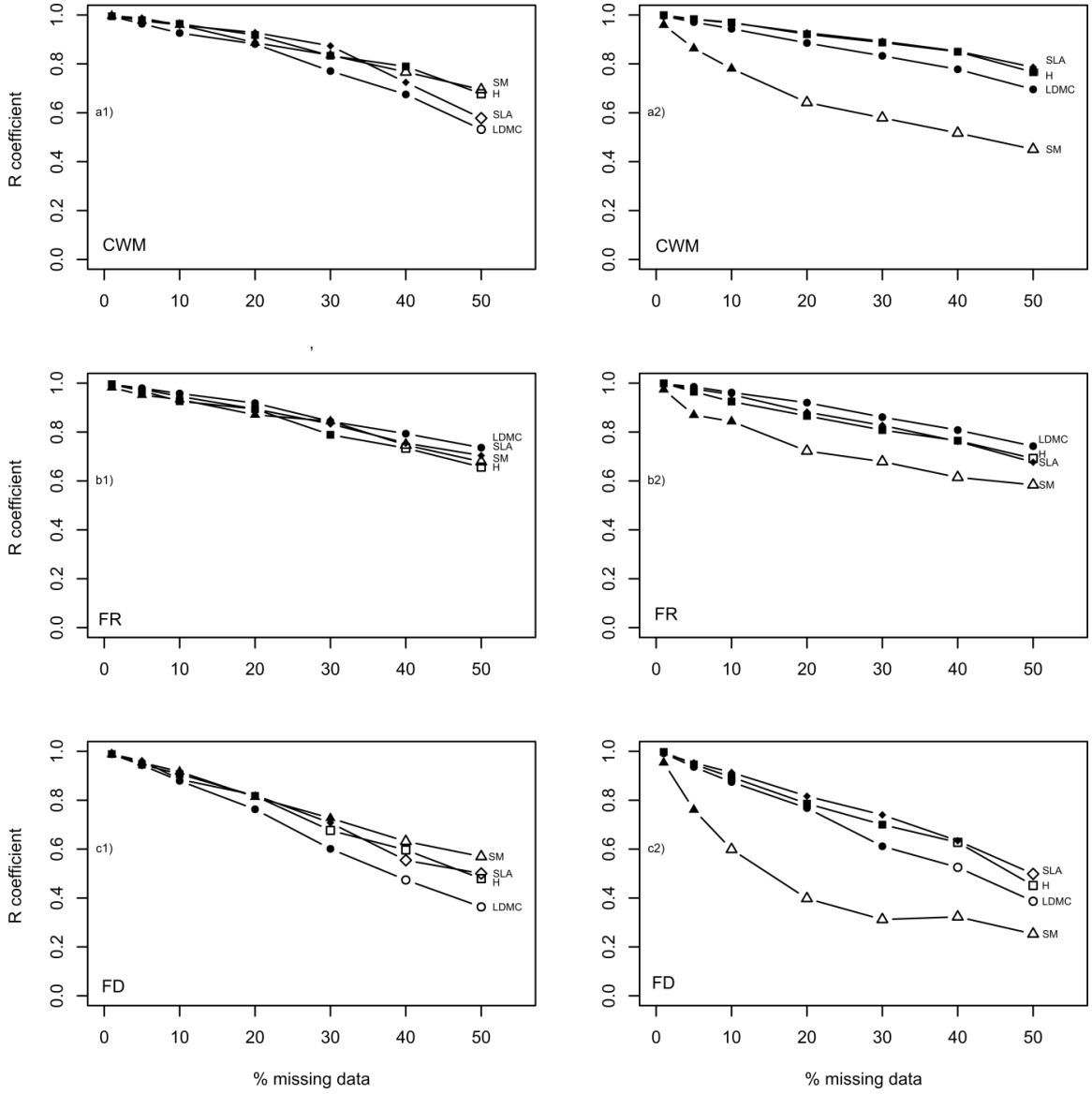

Figure2. 7 Effect of percentage of missing data on the R Pearson coefficient between functional diversity indices calculated without missing data and with imputed data (a) on the community weighted mean (a1 with the dissimilarity method, a2 with the relationships method); b) on the functional range (b1 with the dissimilarity method, b2 with the relationships method); c) on the functional divergence index (c1 with the dissimilarity method, c2 with the relationships method). The formats of the dots represent the functional trait used for the computation of the indices: Triangle for the seed mass (SM), diamond for the specific leaf area (SLA), Circle for the leaf dry matter content (LDMC) and square for the vegetative height (H). Full dots represent levels of percentage of missing data where at least 95% of the correlations were significant (p-value < 0.05). Empty dots represent the simulation where less than 95% of the correlations were significant (p-value < 0.05)

None of these methods was able to perform best for all the traits and indices tested in this study. With the Multivariate Imputation by Chained Equations, the ranking of the grasslands was robust for all indices for the Height, the SLA and the LDMC. But the accuracy of the MICE method was not better than the one of the single imputation methods based on ecological hypothesis for the functional identity and functional richness indices. For the Height, LDMC and SLA, the Relationships method performed as well that the MICE. For the seed mass, the Dissimilarity method was the most accurate for the functional identity and range. Consistently with the results at the species level, the distribution of the trait values seems to be a key parameter in explaining the robustness of the indices to imputation. Indeed, the indices calculated with the seed mass were more robust when imputation was conducted with the Dissimilarity method. The seed mass exhibited an unbalanced distribution in the database with 606 species in contrast to the other traits. The results for the seed mass indicate that the MICE method also has to be used with caution for traits with an unbalanced distribution, although this was not obvious at the species level.
Using the Dissimilarity method for the seed mass (unbalanced distribution) and the Relationships method for the other traits (balanced distribution), the ranking between grasslands remained robust with up to 30% of the data missing for the functional identity (community weighted mean), the functional range, as well as the functional divergence. We propose this percentage of missing data as a limit for the utilization of these single imputation methods. In our simulations, we randomly inserted the missing data by deletion. Each species had thus the same probability to have a missing value. The situation usually encountered in ecological studies is that the most common and dominant species have less missing data than the rare and subordinate species. Indices that are more influenced by dominant species than by minor ones (community mean value and function divergence) might therefore be, for the same percentage of missing data, less affected than in our study. For this type of indices, the 30% threshold is therefore conservative. In grassland plant communities, extreme trait values could be carried by dominant as well as by minor species, so that the effect of the repartition of the missing data is probably unsteady for the functional range index. The errors induced by the imputation of missing values has yet to be compared with other errors, such as those induced by the intraspecific variability of functional traits (Albert *et al.*, 2010a; Albert *et al.*, 2010b).
The threshold of 8 to 19% of missing data for the deletion method, cannot be compared with the 20% of abundance threshold propose by Garnier *et al.* (2004). Indeed, they proposed to measure the functional traits of dominant species only (no traits measured for the minor species). In our study, missing data occurred for both dominant and minor species and could affect one or several traits per

G. Conclusions

At the species level, single imputation methods based on ecological hypothesis and multiple imputation by chained equations induced a lower error on the estimation of missing trait values than imputation by simple average or median computation. At the community level, the error induced by the replacement of missing values with single imputation methods based on ecological hypothesis or with multiple imputation by chained equations when calculating the functional identity, functional range and functional divergence of plant communities is lower than that induced by omitting species with a missing value for a trait. The deletion of species with missing trait values or the utilization of simple imputation methods that do not take the functional differences between species into account (imputation by average or median values), should therefore be avoided prior to the computation of functional diversity indices using trait databases. Single imputation methods based on ecological hypothesis and adapted to the distribution of the trait values can be used instead of multiple imputations by chained equation when studying the ranking of communities for their functional diversity indices. The ranking of plant communities for these functional

diversity indices was not significantly altered by imputing missing values with this method until 30% of the data were missing, as compared with calculation of the indices based on a database without missing data. For future research, improvement in the imputation of missing data in functional trait databases might be achieved by using ecological knowledge in multiple imputation methods.

IV. Effect of the deletion of the minor species in the calculation of functional diversity

A. Objective

In diverse ecosystems such as the permanent grasslands, it is difficult to obtain the functional traits for all the species in the community, especially for the rarer species. For field measurements, simplified protocols are proposed, either considering only dominant species (Garnier *et al.*, 2004), or randomly selecting certain individuals (Gaucherand and Lavorel, 2007). The differences between these simplified protocols and more exhaustive protocols were only tested on the functional identity and dispersion (Lavorel *et al.*, 2008). To our knowledge there are no studies in peer-reviewed literature where the effects of this simplified protocol were tested on the other components of functional diversity.
Even with the utilization of functional trait databases, all the species cannot necessarily be used in the analysis. In botanical surveys, some species are not properly identified; others are not informed in the functional trait database; and for certain species, like trees, the functional traits in the database do not correspond to the same life stage than that of the individuals found in the studied community. Indeed, trees in grasslands are generally at juvenile stage and the measures of functional traits tend to be made on adults. One option is to exclude these species from the analysis. This deletion would however induce a certain level of inaccuracy. **The objective of this study is to test the effect of deletion of less abundant species on the calculation of the different aspects of functional diversity.**

B. Material and methods

Records of minor species were removed from an analysis using data from real grassland plant communities. A set of grassland communities was created by regrouping surveys originating from 3 datasets: one from the Swiss Alps (Peter *et al.*, 2008; Peter *et al.*, 2009), one from the Vosges mountains in North-eastern France (Plantureux and Thorion, 2005) and another from a broader range of regions in France from Atlantic to continental conditions (Michaud *et al.*, 2012b). The grassland surveys used represent a large gradient of ecologic filters (climatic and agricultural management).

Table.2.9 Functional diversity indexes used

Component	Univariate index (u)	Multivariate index (m)
Functional identity (FI)	FI=Community Weighted Mean	/
Functional richness (FR)	FRu :Range of the trait	FRm: FRic index (Villeger *et al.*, 2008)
Functional evenness (FE)	FEu: FROm index (Mouillot *et al.*, 2005)	FEm: FEve index (Villeger *et al.*, 2008)
Functional divergence (FV)	FVu: FDs index (Schleuter *et al.*, 2010)	FVm: FDiv index (Villeger *et al.*, 2008)
Functional dispersion (FD)	FDu: FDis index (Laliberté and Legendre, 2010)	FDm: FDis index (Laliberté and Legendre, 2010)

Our first attempt only used botanical surveys in which the functional traits were informed for 100% of the surveyed species (excluding tree species), and where all species were present in the functional trait databases with at least one trait value. However, too few botanical surveys with these conditions were available. We therefore took all the surveys where the sum of the relative abundance of the species with missing information was less than 5%. Using these criteria, 722 surveys were available with 606 species. The functional traits were extracted from the LEDA trait database

(Kleyer *et al.*, 2008). We limited the trait selection to 4 traits (specific leaf area SLA, seed mass SM, vegetative height H, and leaf dry matter content LDMC) often used in grassland studies: The SLA, H and SM are for instance the traits proposed in the leaf-height-seed (LHS) model (Westoby *et al.*, 2002). Moreover, LDMC and SLA are important traits in the leaf economic spectrum (Wright *et al.*, 2004). These two traits are very often linked with ecosystem functioning. The missing data, on the species with a least one value, we corrected using the dissimilarity method presented in the part II of this chapter.
For the selected functional diversity, we chose different types of indexes for each component of functional diversity, expect for the functional identity: a one-dimensional index and a multidimensional index. These univariate or multivariate indexes are presented on Table 2.9. One-dimensional indexes, i.e. univariate indexes were computed for each trait and multidimensional indexes, i.e. multivariate indexes on the four traits together.
From the pool of 722 surveys, we randomly selected 50 surveys. The selection of the 50 surveys was repeated 100 times. The deletions of minor species were made on these communities.
For a given community, we deleted the least abundant species in the botanical samples. For the deletion, the species were progressively deleted beginning with the less abundant species, then the second less abundant, etc. The deletion stopped when the next deletion would induce a deletion of abundance superior to a threshold (tab). For example, for a threshold of 10%, the deletion of species was never superior to 10%. The deletion of species was made with different abundance threshold from 1 % to 40% by step of 1%.
The deletions were made on these 50 surveys for 100 sets of surveys. The functional diversity indexes, computed on surveys with the deletion, were compared with the functional diversity computed on surveys without the deletion. The p-value and the R Pearson coefficient were calculated. For each threshold of abundance deleted, we counted the number of sets (of the 100 different set of 50 communities) where the p-value between the functional diversity computed with the deletion and without deletion was not significant. By linear interpolation between the threshold of deleted abundance and the percentage of non-significant correlation, we estimated the percentage of deleted abundance where 5 of the 100 sets of communities had no significant correlation p-value. We considered this point to be the threshold for maximum deletion while still maintaining an acceptable level of accuracy. We assumed thereafter that the deletion of minor species was acceptable if the deleted abundance did not exceed this threshold

C. Results

The results of the simulation are presented in Figure 2.8 and Table 2.10. The functional identity was always robust to the deletion of minor species. On the other hand, functional evenness components were strongly affected by the deletion of species starting from the first % of abundance deleted. For the divergence, the calculations were robust until 30% of deleted abundance for the univariate indexes (31% for H, 36% for LDMC, 27.33% for SM and 35.50 % for the SLA). The multivariate divergence was more affected that the univariate (7.25%). For the functional dispersion indexes, the thresholds were around 35% (35.67% for H, 35.60 for LDMC, 36.50 for SLA, 35.50% for SM, 37.75 for the multivariate). For the functional richness, the results differed between the traits. The functional richness of SM was very sensitive to the deletion (1%), the specific leaf area (7.5%), the vegetative height (12%) and the leaf dry matter content (22%). The multivariate functional richness threshold had a robustness threshold of 2.25%.

D. Discussion

Functional identity can be estimated based on only the abundant species of a community (here 60% of the abundance). These results are consistent with other studies (Garnier *et al.*, 2004; Lavorel *et al.*, 2008). The calculation of the community weighted mean value depends only on the dominant species and so the minor species can be eliminated.
Many functional identities are characteristic of grasslands. The classifications between grasslands in terms of functional identities are often very robust. Functional divergence and dispersion also mainly relied on the dominant species (Rao, 1982; Mason *et al.*, 2003; Villeger *et al.*, 2008; Laliberté and Legendre, 2010). These indexes are robust to the deletion of the minor species.

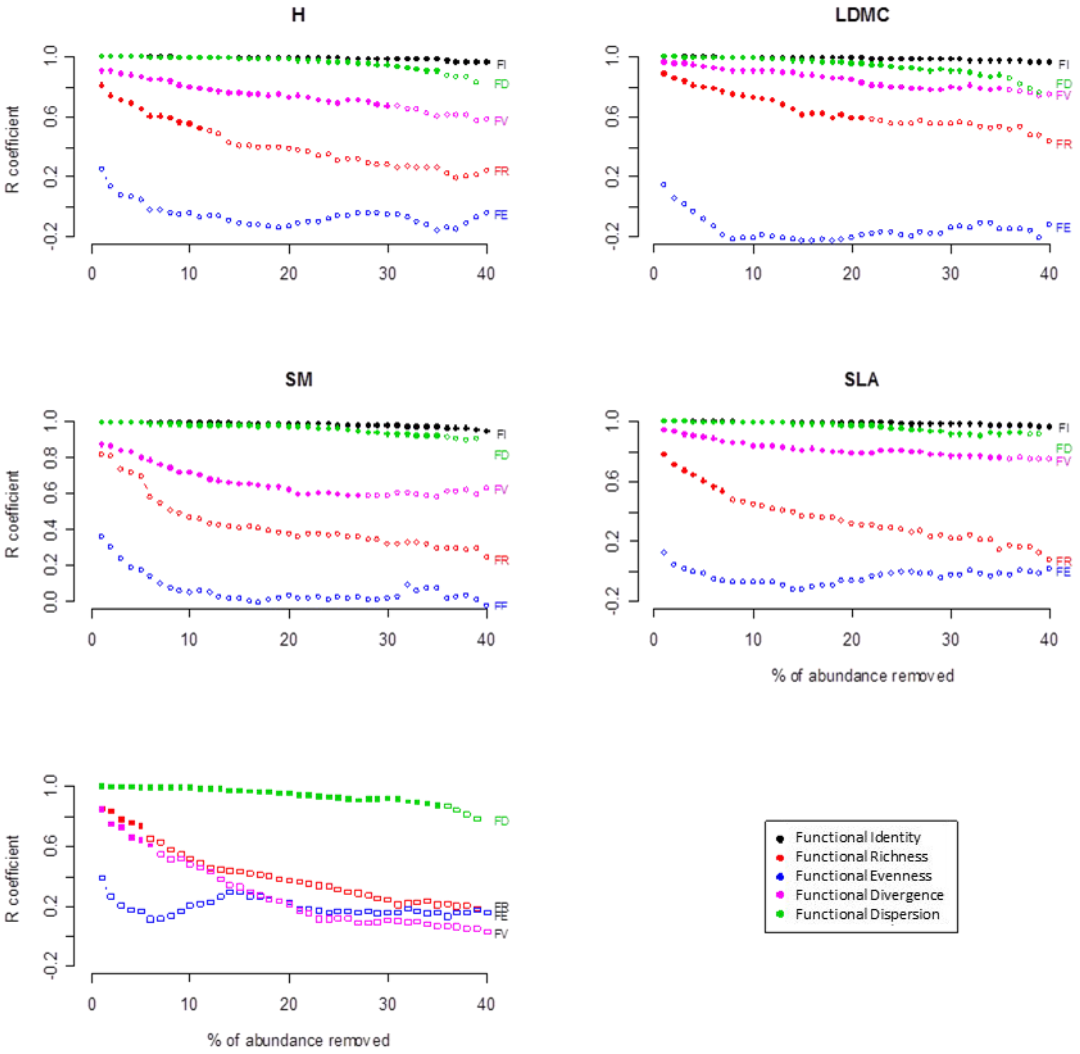

Figure.2.8: Effect of deletion of species on the R Pearson coefficient between Functional diversity indexes before and after the deletion of species. a) Univariate index computed with vegetative Height, b) univariate index compute with LDMC c) univariate index computed with SM, d) univariate index computed with SLA e) multivariate index computed with the 4 trait. Each point is the average R coefficient of 100 simulations (100 sets of 50 communities). Full dots represent levels of intraspecific variability where at least 95% of the correlations were significant (p.value<0.05). Empty dots represent the simulations where less than 95% of the correlations were significant (p.value<0.05).

The functional evenness relies on all the species of the community; therefore, the deletion of one species has a strong effect on the outcome of the calculation (Mouillot *et al.*, 2005; Villeger *et al.*, 2008). It is difficult to accurately calculate the functional evenness without all the species in the community.

The functional richness relies on the extreme values of the functional traits (Petchey and Gaston, 2002; Cornwell *et al.*, 2006). Our results show that functional richness can be calculated with the deletion of the minor species. This suggests that the extreme values of functional traits are not associated with the minor species in a community. The difference of results between different traits also suggests that the dominance of species with extreme values varies from trait to trait. For example, we can suppose that for the seed mass, the extreme values are associated with less abundant species. On the other hand, the extreme value of LDMC seems to be associated with more abundant species (Table.2.10). The distribution of the trait value is an important factor within the community.

Table.2.10: Abundance threshold to the deletion of less abundant species for the different indexes of functional diversity.

component	Trait	Robustness threshold of the deletion of species in %
FI	H	=<40.00
FI	LDMC	=<40.00
FI	SM	=<40.00
FI	SLA	=<40.00
FR	H	12.00
FR	LDMC	22.00
FR	SM	1.00
FR	SLA	7.50
FR	MULTI	2.25
FE	H	1.00
FE	LDMC	1.00
FE	SM	1.00
FE	SLA	1.00
FE	MULTI	1.00
FV	H	31.00
FV	LDMC	36.00
FV	SM	27.33
FV	SLA	35.50
FV	MULTI	7.25
FD	H	35.67
FD	LDMC	35.60
FD	SM	35.50
FD	SLA	36.50
FD	MULTI	37.75

In conclusion, functional identity and divergence can be easily calculated in surveys where less abundant species are removed. For functional richness, the deletion of abundant species can be problematic for certain traits (seed mass for example). Many studies have tested the effect of environment on the selection of functional either at the individual scale or species scale (Diaz *et al.*, 2007b; Ordoñez *et al.*, 2009). Few studies show the effect of the environment on the distribution of abundance inside the functional space. Overall, the distribution of the abundance inside the functional space is not often studied. Further study of the evenness or divergence would be useful to understand the determinisms of the distribution of abundance in the functional trait space.

V. Functional diversity-area relationship in permanent grassland

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A. Abstract

The target of a multi-functional, sustainable use of grasslands requires deeper understanding of the relationships between grassland management and plant diversity and its consequences on ecosystem functioning. Some of these relationships are well known for specific pedo-climatic conditions. One option to extend our knowledge to a broader range of conditions is to aggregate different studies by performing a meta-analysis. Nevertheless, differences in sampling area between studies are a major challenge. For taxonomic diversity, species-area curves have been established for different habitats but references for functional diversity are scarce. We aimed at assessing the possibility of using functional diversity-area curves to correct for differing sampling area between studies before merging data for overall analysis on functional diversity. We measured diversity in 9 nested areas of increasing size (from 0.01 m² to 100 m²) in 16 grasslands with different management (grazing, mowing).We analyzed the effect of area on specific richness, functional richness, functional divergence and community weighed mean value of several functional traits (SLA, LDMC) and on the percentage of legumes. We conclude that correction for differing sampling area is possible for functional richness and community weighted mean values.

Keywords: plant functional traits, functional diversity, biodiversity-area relationships, survey minimal area.

B. Introduction

One argument for the preservation of permanent grassland in ruminant based farming systems is the provision of several ecosystem services. Ecosystem services (ES) are the services provided by the ecosystem for mankind. ES can be linked to some functional diversity criterion (de Bello *et al.*, 2010). Functional diversity (FD) is the set of values of a functional trait (or multiple traits) of the individuals (or species) of a community. Functional diversity is driven by the environmental conditions (agricultural practices and pedo-climatic conditions). The relationships between FD and ES and between FD and environmental factors are generally studied only for local conditions. The generalization of these relationships has to be made at larger scales. One solution is to gather the different local studies. The main problem is the differences of survey protocol between studies, especially in term of the sampling area of the survey. Our goal is to assess the relationships between sampling area and some measures of functional diversity in order to correct for differing sampling area between studies.

C. Materials and Methods

We studied 16 permanent grasslands with contrasting management conditions in North-eastern part of France in 2010. The botanical composition was recorded on 9 different nested square areas (0.01 m², 0.0625 m², 0.25m², 0.5625m², 1m², 6.25m², 25m², 56.25m², 100m²). For quadrates below 1m², the abundances of species were visually estimated over the whole surface. For surfaces greater than 1m², the abundances were visually estimated by subsampling several quadrates of 0.25m². The

number of quadrates was proportional to the surface (2 for 6.25 m², 4 for 25 m², 7 for 56.25 m², and 11 for the 100m²). Different plant diversity criterions were considered: the total number of species, the percentage of legumes in the sward, the aggregated Trait of Leaf Dry Matter Content (LDMC) and Specific Leaf Area (SLA). The aggregated trait is the sum of the trait of each species weighted by its relative abundance. It represented the average trait of the community. We also studied the functional amplitude of LDMC and SLA, as the difference between the minimum trait value and the maximum trait value of the community and the Rao index of these two traits. The values of the functional traits per species were taken from the LEDA trait database (Kleyer *et al*., 2008). These criteria were calculated using the FD package on R 2.13.1(Laliberté & Legendre 2010). The links between biodiversity criterion and the sampling area was studied using the model Criterion =a*log (area) +b. We calculated the area required to detect ± 5% of the diversity (minimal area) by linear interpolation between the measures. If the value of the diversity criteria was increasing with the sampling area, the minimal area was considered as the smallest area with 95% of the biodiversity of the 100 m² area. For diversity criteria values decreasing with the sampling area, the area with 105% of the biodiversity of the 100 m² area was considered minimal.

D. Results

We found a close relationship between the sampling area and the number of species, the functional amplitude of SLA and LDMC (Figure 2.9). For the other diversity criteria, no relationships with the area were found. The two aggregated traits have the smaller minimal area among all indexes (around 2 m²). The other indexes have a minimal area bigger than 10 m² (Table 2.11).

Table 2.11: Relationships between area and biodiversity (AT: aggregated trait, FA: functional amplitude, ns p-value no significant, * p-value significant)

Diversity criterion	**Minimal area(m²)**	**Overall Model**	**R_2**
Number of species	59.16	1.49 *log(Area)+15.35	0.35*
% legumes	54.9	-1.0*log(Area)+21.99	0.05 ns
Aggregated Trait of LDMC	1.61	0.23*log(Area)+ 214.87	0.003 ns
Aggregated Trait of SLA	2.09	-0.10*log(Area)+ 26.59	0.02 ns
FA LDMC	26.2	0.028*log(Area)+ 0.679	0.23*
FA SLA	14.25	0.015*log(Area)+ 0.694	0.11*
Rao LDMC	31.45	0.00*log(Area)+ 0.481	0.00ns
Rao SLA	55.72	0.015*log(Area)+ 0.694	0.00ns

E. Conclusion

Functional amplitude and the number of species can be related to the sampling area. A model of these relationships can be used to match data coming from various origins, differing in the sampling areas. For the aggregated traits, the minimal area is very small. All studies with a sampling area greater than 2 m² can be aggregated without correction. For the other indexes (Rao and % of legumes), the minimal area is large and no relationship with the sampling area was found. Thus harmonization for these

indexes seems impossible. This preliminary work shows some ways to deal with difference in survey protocol for meta-analysis. However further studies with a larger number of grasslands in a wider range of conditions should be performed to ascertain these conclusions.

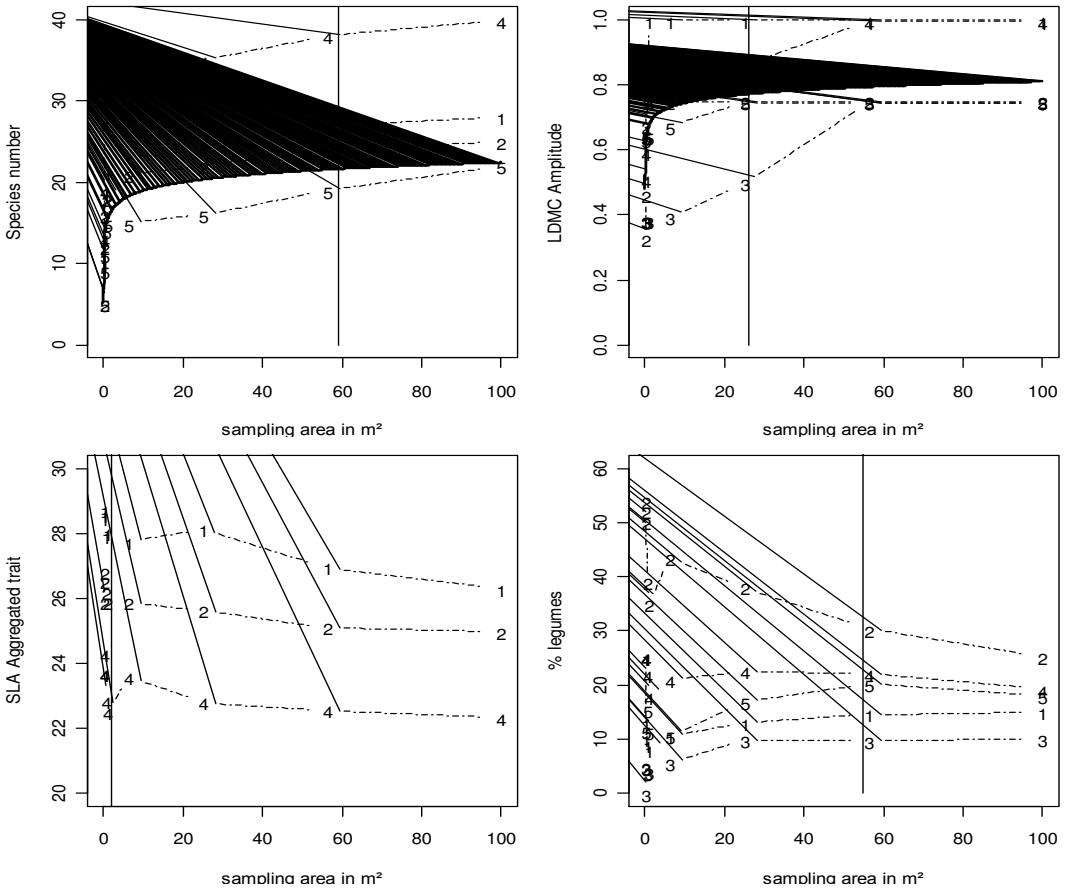

Figure2. 9: Evolution of selected biodiversity criteria with sampling area for 5 grasslands. The vertical line represents the minimal area and the bold curve represents the overall model a) relationship with the number of species b) With the LDMC amplitude c) with the SLA Aggregated trait d) the percentage of legumes.

VI. Chapter Discussion

A. Difference between the components of functional diversity

As a result of the different methodological studies performed in this chapter, we can propose a level of confidence for the calculation of functional diversity for the different functional diversity criteria proposed in the first chapter. Five different sources of errors were assessed: intraspecific variability, missing data in functional trait database, deletion of minor species, sampling area and method of estimation of abundance (Table.2.12). For the protocol of estimation of abundance, we used the conclusion of previous works (Gaucherand and Lavorel, 2007; Lavorel *et al.*, 2008). For the criteria based on functional traits, the level of confidence was estimated for the 5 sources of error. For the criteria based on taxonomical or functional groups, we only propose levels of confidence regarding the sampling area and the method of estimation of abundance.

Functional identity was the functional diversity component less influenced by the different errors (intraspecific variability, missing data, and deletion of subordinate specie and area of survey.) Two explanations of this robustness to the different errors: firstly the CWM only relied on dominant species and more information is available for dominant than for minor species. Secondly, community weighted mean values were very variable between the communities in our dataset. The robustness of CWM is concordant with other studies (Lavorel *et al.*, 2008; Albert *et al.*, 2011; Albert *et al.*, 2012). Functional identity was also robust against different methods of estimation of abundance (Lavorel *et al.*, 2008).

For the functional richness, the indexes were robust to the intraspecific variability of functional traits and to the imputation of missing data. But functional richness was affected by the deletion of subordinate species (depending on the functional trait) and by the sampling area. The functional richness indexes are not calculated using abundance information, so the method of estimation of abundance is of no importance in this case.

For the functional divergence and dispersion, the indexes were robust to the imputation of missing data and to the deletion of minor species. However, the other types of errors had a strong effect on functional divergence (intraspecific variability and sampling area).

The **functional evenness** was robust to the intraspecific variability and to the imputation of missing data. Functional evenness was, however, sensitive to the sampling area and the deletion of minor species.

For the other criteria, the ones that relied on an abundance of a plant group (taxonomical or functional groups) were strongly affected by the sampling area. We can suppose that these criteria are also strongly affected by the method of estimation of the abundance. Because the relative abundance of grasses was in the vast majority of the grasslands of the database much larger than the relative abundance of the legumes, the estimated percentage of legumes was probably more affected by these two errors than the percentage of grasses.

The number of plant species and the other criteria that relied on the count of groups were only affected by the sampling area.

The effects of the intraspecific variability, of missing data in functional trait database, of the deletion of minor species and of the sampling area were studied separately in the previous parts of this chapter. However, these four sources of errors might interact together and influence the calculation of functional diversity together.

Table.2.12: Level of confidence on the calculation of the different criteria of vegetation for the different error tested in this chapter. ITV: effect of intraspecific variability, NA: the effect of the imputation of missing data, Deletion: Deletion of minor species in surveys, Area: effect of the survey area and Abundance: method of estimation of the abundance. For the abundance, we extrapolated results from previous studies. The level confidence was categorized in +/=/- confidence. The / was wrote in the case where the error has not to be considered. See the legend of Table.1.4 for the explanation of the abbreviation of the functional criteria.

Functional Criteria	**ITV**	**NA**	**Deletion**	**Area**	**Abundance**	**Total**
%forbs	/	/	/	-	=	=
% Grass	/	/	/	=	=	=
% legumes	/	/	/	-	=	=
Legumes grass	/	/	/	-	=	=
% apiaceous	/	/	/	-	=	=
%dicot	/	/	/	=	=	=
%Bee sp	/	/	/	-	=	=
%Bumblebeessp	/	/	/	-	=	=
% Butterflysp	/	/	/	-	=	=
% Bumblebeessp legumes	/	/	/	-	=	=
Beenbsp	/	/	/	=	/	+
Bumblebeesnbsp	/	/	/	=	/	+
Butterflynbsp	/	/	/	=	/	+
Number sp	/	/	/	=	/	+
CWMH	+	+	+	+	+	+
CWMLDMC	+	+	+	+	+	+
CWMLNC	+	+	+	+	+	+
CWMOFL	+	+	+	+	+	+
CWMSLA	+	+	+	+	+	+
FDH	-	+	+	-	=	=
FDSLA	-	+	+	-	=	=
FEH	+	+	-	-	-	=
FRH	=	=	=	=	/	=
FRLDMC	=	=	=	=	/	=
Ellenberg Temperature	/	=	=	+	+	+
Humidity Ellenberg	/	=	=	+	+	+
Flower duration	-	-	=	-	=	-
Nectar quantity	-	-	=	-	=	-
Pollen Quantity	-	-	=	-	=	-

B. Conclusion

These different studies show that some functional diversity criteria can be calculated using functional traits databases and a set of botanical surveys with a good level of confidence, despite on the sources of errors due to intraspecific variability, missing data in the traits database or differences in sampling area or method of estimation of abundance between the surveys. Nevertheless, the calculations of other functional diversity criteria are so sensitive to these sources of errors, that the possibility of utilizing them in further analysis seems very limited.
This level of confidence was afterwards used to evaluate the quality of the analysis in the rest of the manuscript.

Chapter 3: Effects of management and climate on plant functional diversity in permanent grasslands and estimation of plant functional diversity from climate and management.

Abstract chapter 3

The objective of this chapter is to evaluate the dependence of functional diversity criteria on management and climate, in order to use these variables to estimate functional diversity criteria. We tested two types of relationships: general trends and conditional effects. The general trends were assessed using structural equation modeling. Conditional trends were tested using regression trees and random forests. A dataset of 439 grasslands in France and Switzerland with 19 different climatic and management variables was used. The dataset covered a wide range of management and climatic conditions. The climatic variables were found to have more effect on the functional diversity than management. Estimation of the functional diversity criteria with conditional effects led to more accurate than results than with general trends. Such estimations can subsequently be used in ecosystem service evaluation tools.

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I. Context /Objective

The first objective of this chapter is to evaluate the dependence of plant functional diversity criteria to management and climate. We then use this evaluation to test the accuracy of predicting these criteria using climate and management variables. Such estimations can subsequently be used in ecosystem service evaluation tools.

We first performed a review of the known effects of management and climate on functional diversity of grasslands. This review is described in the deliverable D3.2 "Progress report on the effect of grassland management on grassland biodiversity from the field to the landscape level as input for Task 3.4 and 3.5." from the MULTISWARD project (www.multisward.eu). This document is presented in Annex I. We will present some information coming from this document in order to introduce the work and justify some choice in the analysis performed.

Functional diversity is a result of the effects of ecological filters on species selection (Keddy, 1992; McGill *et al.*, 2006). For example, specific leaf area is influenced by climatic factors such as precipitation (Wright *et al.*, 2001; Ackerly and Cornwell, 2007). Specific leaf area and the other leaf economic spectrum functional traits are influenced by soil fertility (Ordoñez *et al.*, 2009). Disturbance, such as grazing, also affects specific leaf area (Diaz *et al.*, 2001; Diaz *et al.*, 2007b). The onset of flowering is influenced by climate and disturbance (Michaud *et al.*, 2012b).

The effects of management and climate on functional diversity at the community level can be scaled up from their effect on the individual plant. At the community level, functional identities of leaf economic spectrum traits are affected by soil fertility and disturbance intensity (Louault *et al.*, 2005; Garnier *et al.*, 2006; Lavorel *et al.*, 2011; Laliberté *et al.*, 2012; Lavorel and Grigulis, 2012). Functional richness generally decreases with management intensification (Flynn *et al.*, 2009; Pakeman, 2011). Some studies show that functional divergence follows a humped-shape curve along the management intensity gradient and soil depth. Indeed, the highest functional divergence was found for medium management intensity and medium soil deepness (Bernard-Verdier *et al.*, 2012; Duru *et al.*, 2012b).

These results on functional diversity are generally obtained from experimental sites (Louault *et al.*, 2005; Bernard-Verdier *et al.*, 2012; Laliberté *et al.*, 2012) or from studies at small spatial scales (Garnier *et al.*, 2004; Lavorel *et al.*, 2011). Generally, the number of studied factors is low and therefore only few ecological filters are tested in each study. However, the functional traits and their diversity are driven by a large set of factors. In the ecological filters theory (Keddy, 1992), functional traits are first selected by climate, then soil, then disturbance and finally by competition. The management and the climate might therefore be used to estimate functional diversity. Very few studies have tried to test the capacity of management and climate to estimate functional diversity criteria (Michaud *et al.*, 2012b).

In order to estimate plant functional diversity, studies with large climatic and management gradients are necessary. Indeed, the evaluation of the relative strength of each filter and, eventually, identifying missing factors would be important for the estimation of plant functional diversity.

Furthermore, large scale studies could be used to evaluate the interactions between the different ecological filters. For example, the effect of defoliation on functional diversity depends on climatic conditions: grazing was found to increase plant functional diversity under dry conditions (de Bello *et al.*, 2005a, 2006). Conversely, functional diversity decreased with grazing intensity in wetter conditions (de Bello *et al.*, 2005a, 2006). This "conditional effect" is due to the multiple functional strategies selected by grazing: for example, grazing-tolerant species are able to recover quickly after defoliation (high SLA) while grazing-resistant species protect themselves (chemical or physical defenses i.e. high LDMC) (Diaz *et al.*, 2007b). In wet and fertile conditions that favor high SLA strategies, grazing tolerant species are more successful due to high levels of defoliation (de Bello *et al.*, 2005a). In dry or nutrient-poor conditions, grazing-tolerant species are less competitive and so cohabit with the grazing-resistant species.

Both of the strategies are favored by grazing (Vesk *et al.*, 2004; de Bello *et al.*, 2005a). This is one example of the successive effects of ecological filters on functional traits (Keddy, 1992). Indeed, the functional traits are first influenced by climate and soil fertility. Secondly, grazing selects some of the remaining trait values. The effects of grazing are in this case dependent of the climate condition.
Regarding the numerous effects reported in the literature, we hypothesize that management and climate can be used to estimate functional diversity criteria.
In current literature, very few attempts to estimate plant functional diversity using management and climate have been made. We therefore had to develop our own method of estimation. We used real data to develop statistical analysis to evaluate the links between functional diversity, management and climate. The few examples presented above also show that the relationships between functional diversity and climate and management can follow "general trends" (effects of a variable independent of the values of the other values) or follow "conditional effects" (effects of a variables depending on the value of other variables). We tested the two types of effects.
In order to separate the effect of climate and management, we used a dataset with a large number of plots. We obtained this dataset by combining different datasets (three datasets already published; see paragraph II).

II. Presentation of the dataset

A. Datasets used and description of the vegetation

We used three datasets to test the relationships among functional diversity, management and climate. At the beginning of the Multisward project, we wanted to use more datasets from more different countries than France and Switzerland. However, the different datasets of the MULTISWARD project partners were either not available or did not contain all the needed information. Thus, we only used data from France and Switzerland. The combination of these three datasets covers a large diversity of climate conditions, management and botanical composition as presented in the following paragraphs.
The vegetation aspects of the datasets are presented in this section (II.A). The climate and the management aspects are presented in the next section (II.B).
The first dataset contains surveys of 140 permanent grasslands from all regions of France except of the Alps and Mediterranean regions. This dataset was originally developed to create a French national typology of permanent grasslands (Michaud *et al.*, 2011) and to assess the relationships between services, vegetation and management. We thereafter name this dataset the **French national typology dataset**. To establish this set, farmers were first interviewed by experts about the management of more than 1500 geo-referenced plots. From this information, 140 grasslands were chosen in order to take into account the management and the pedo-climatic gradient observed in the 1500 plots. The occurrence of all plant species was registered on the whole parcel area. The abundance of the species was determined in 8 quadrats of 0.5m x 0.5m (Michaud *et al.*, 2012b). Surveys were performed in 2009 by several botanical experts.
Another dataset contains 70 permanent grasslands from the Massif Vosgien (Eastern France Mountains). The aim of this dataset was to build a grassland typology for this region (Plantureux and Thorion, 2005). In this dataset, grassland altitude ranged from 250 to 1200m asl, and various management practices are represented (meadows, grazed grasslands …). We thereafter name this dataset **the Massif Vosgien dataset**. The presence of plant species was registered on the whole plot area and their abundance was assessed by 25 handles in the plot (1 to 4 ha). Surveys were performed in 2004 by the team of Laboratoire agronomie environnement (UDL-INRA Nancy-Colmar).

The third dataset consists of 229 permanent grasslands in the Swiss Alps. The objective of this study was to observe the change in plant diversity over 20 years in different regions of the Swiss Alps (Peter *et al.*, 2008; Peter *et al.*, 2009). We thereafter name this dataset **the Swiss Alps dataset**. Surveys were made on plots where historic surveys were available. The botanical composition was surveyed on 5m x 5m observation plots. The species abundance was estimated using the Braun-Blanquet method. In this dataset, plots used for forage conservation are more frequent than grazed plots because summer pastures were not included in the survey.
In order to regroup the botanical surveys among the three datasets, the nomenclature was harmonized using the LEDA systematic (Kleyer *et al.*, 2008). Functional traits were extracted from the LEDA database (Kleyer *et al.*, 2008) for the vegetative height(H) and from the TRY database (Kattge *et al.*, 2011) for the specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC) and onset of flowering (OFL). Missing values were imputed using the relationships method for the different traits (see chapter 2.I for the description of the imputation's methods). Botanical families and traits regarding pollination were extracted from the e-FLORA-sys database (Plantureux and Amiaud 2010) and from the Floraweb database (http://www.floraweb.de). The tree species, the unidentified species, as well as the species without any functional trait values in the trait database were deleted from the survey. The species without any traits in the functional traits database were kept only for the computation of number of species and botanical family percentage.
The 29 functional diversity criteria selected in the first chapter were then computed. The functional richness was calculated using the differences between the minimum and the maximal value of the functional trait in the community (Mason *et al.*, 2003). The functional dispersion was computed with the FDisp index (Schleuter *et al.*, 2010) and the functional evenness with the FROm index (Mouillot *et al.*, 2005).
For the computation of the functional diversity criteria, we decided not to correct the data due to the differences in protocol between the surveys. Indeed, the analysis performed in chapter 2 showed that functional identity criteria were insensitive to the sampling area and that the increase in the values for species and functional richness was small from a sampling area of 25 m^2 (smallest survey area in the dataset). For the plant species richness, this was in agreement with previous studies (Dierschke, 1994). For functional dispersion criteria and the relative abundance of legumes an irregular relationship with the sampling area was found, but no correction could be proposed. To take this source of error in consideration, the origin of the surveys (the three datasets) was included as a variable in some of the analyses made in this chapter.
The distributions of some of the functional diversity criteria in the three datasets are presented in the Figures 3.1 and 3.2. The average, minimum, maximum, standard deviation of the 29 different functional diversity criteria on the dataset obtain with the combination of the three different datasets are presented in Table 3.1.
For the rest of the chapter, we mainly focus our work on seven functional diversity criteria in the list: the number of plant species, the community weighted mean value of SLA, LNC, OFL and H and the functional dispersion of SLA and H. For the other criteria, we estimated the accuracy of the analysis.

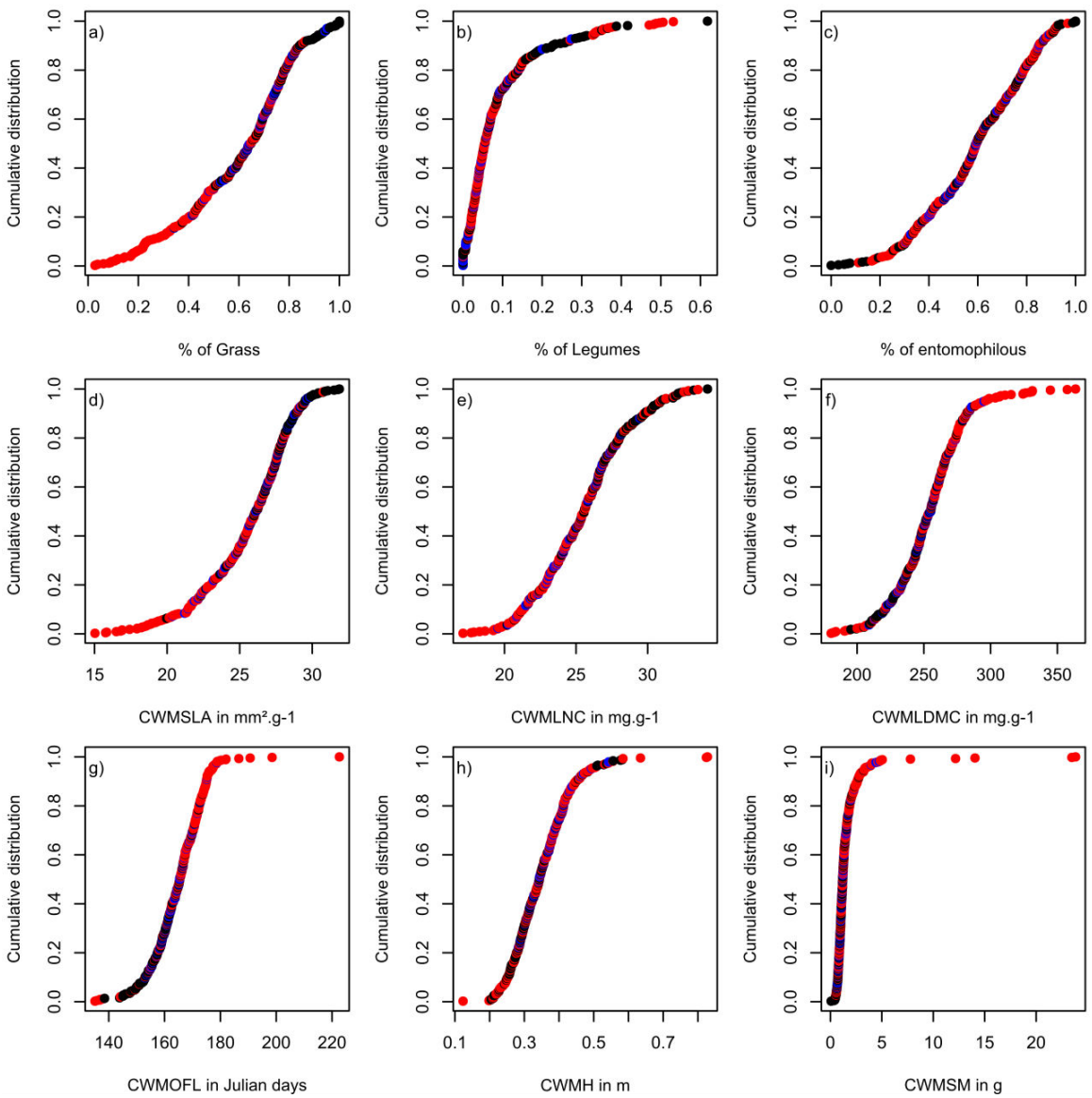


Figure.3.1: Cumulative distribution of a set of functional diversity criteria in the three datasets: a) percentage of grass species, b) percentage of legumes, c) percentage of entomophilous species, d) community mean value of specific leaf area, e) community mean value of leaf nitrogen content, f) community mean value of leaf dry matter content, g) community mean value of onset of flowering, h) community mean value of height and i) community mean of seed mass. In black the French typology dataset, in blue the Massif Vosgien dataset and in red the Swiss Alps dataset

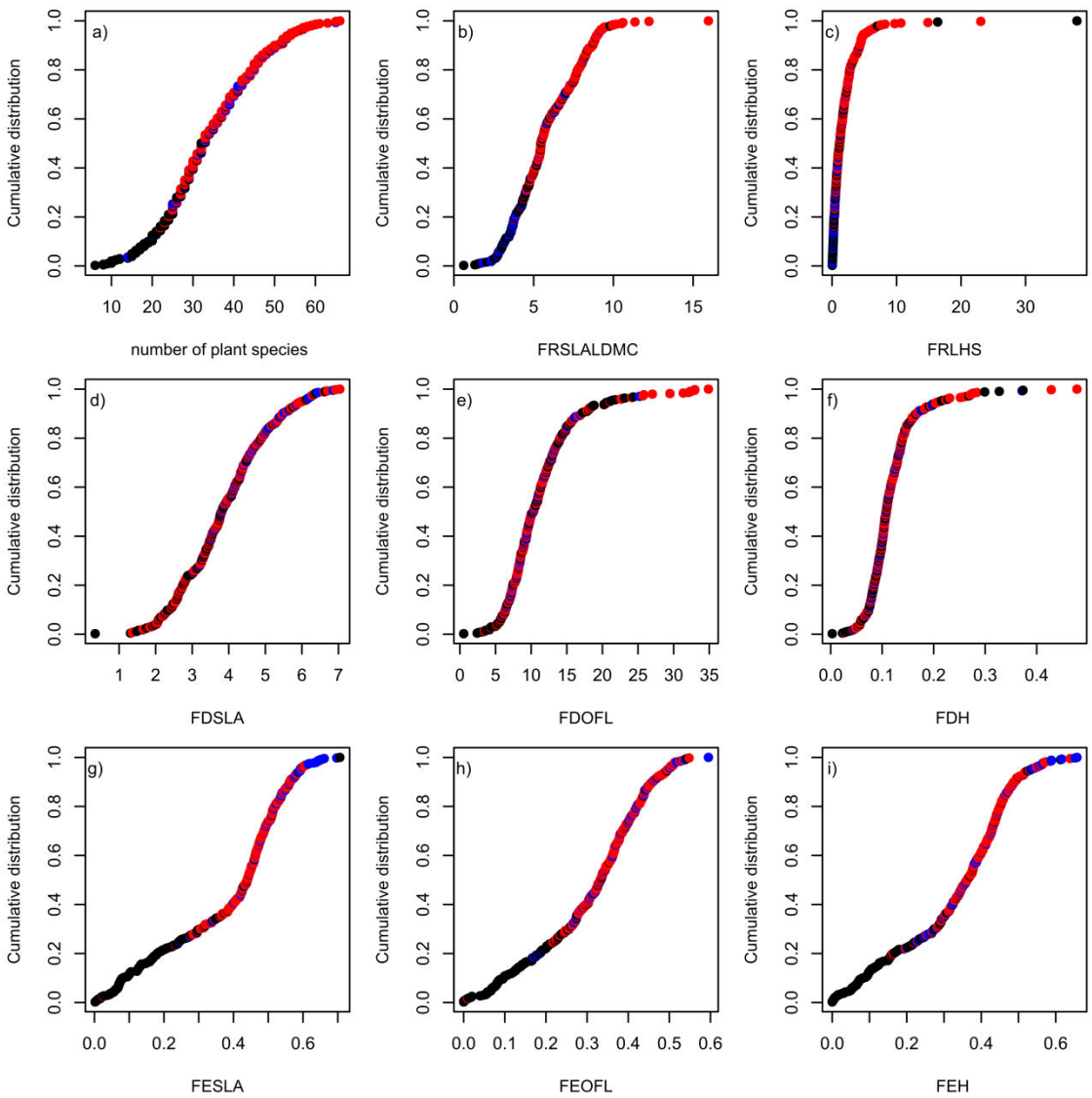


Figure.3.2: Cumulative distribution of functional diversity criteria in the three datasets: a) number of plant species, b) Functional richness of SLA and LDMC, c) Functional richness of SLA, H and SM (Leaf-Height-Seed), d) Functional dispersion of specific leaf area, e) Functional dispersion of onset of flowering, f) Functional dispersion of height, g) functional evenness of specific leaf area, h) functional evenness of onset of flowering and i) functional evenness of vegetative height. In black the French typology dataset, in blue the Massif Vosgien dataset and in red the Swiss Alps dataset.

Table.3.1: List of the functional diversity criteria studied with the distribution of their values in the datasets (mean, standard deviation, minimal and maximal value). The functional diversity criteria in bold are the criteria more studied in the chapter.

Abbreviation	Name	Average	SD	Min	Max	Unit
%forbs	Relative abundance of forbs	0.31	0.21	0	0.93	%
% Grass	Relative abundance of grass	0.60	0.22	0.03	100	%
% legumes	Relative abundance of legumes	0.09	0.10	0	0.62	%
Legumes grass	Ratio legumes on grass	0.27	0.69	0	10.12	
% apiaceous	Relative abundance of apiaceous	0.06	0.10	0	0.55	%
%dicot	Relative abundance of dictoyledons	0.40	0.22	0	0.97	%
%Bee sp	Relative abundance of species pollinated by bee	0.13	0.12	0	0.71	%
%Bumblebeessp	Relative abundance of species pollinated by bumblebees	0.09	0.12	0	0.66	%
% Butterflysp	Relative abundance of species pollinated by butterflies	0.05	0.04	0	0.53	%
% Bumblebeessp legumes	Relative abundance of legumes species pollinated by bumblebees	0.01	0.04	0	0.50	%
Beenbsp	Number of species pollinated by bees	7.33	4.39	0	20	species
Bumblebeesnbsp	Number of species pollinated by bumblebees	5	3.79	0	17	species
Butterflynbsp		1.79	0.97	0	5	species
NBSP	**Number of plant species**	**33.93**	**12**	**6**	**66**	**Species per plot**
CWMH	**Community weighted mean value of H**	**0.35**	**0.09**	**0.12**	**0.82**	**m**
CWMLDMC	Community weighted mean value of LDMC	254.01	26.55	180.54	363.62	mg g^{-1}
CWMLNC	**Community weighted mean value of LNC**	**25.6**	**3.2**	**17.1**	**34.2**	**mgg^{-1}**
CWMOFL	**Community weighted mean value of OFL**	**164.8**	**9.28**	**135.1**	**222.6**	**Julian days**
CWMSLA	**Community weighted mean value of SLA**	**25.5**	**3.1**	**15.0**	**31.9**	**mm^2mg^{-1}**
FDH	**Functional dispersion of H**	**0.11**	**0.05**	**0.00**	**0.47**	
FDSLA	**Functional dispersion of SLA**	**3.9**	**1.20**	**0.34**	**7.0**	
FEH	Functional evenness of H	0.33	0.15	0	0.66	
FRH	Functional richness of H	0.91	0.28	0.27	2.34	m
FRLDMC	Functional richness of LDMC	244.90	43.45	90.96	379.51	mg g^{-1}
Ellenberg Temperature	Temperature Ellenberg index	4.54	0.84	2.73	6.33	
Humidity Ellenberg	Temperature Ellenberg index	5.31	0.52	3.77	7.17	
Flower duration	Flower duration	4.44	1.47	0	8.00	month
Nectar quantity	Quantity of nectar	7	0.6	0	8	score/10
Pollen Quantity	Quantity of pollen	8.68	1.64	0	9	score/10

For most of the 29 functional diversity criteria, the standard deviation was relatively high (Table 3.1). We assumed that our dataset covered large gradients in terms of plant biodiversity (functional and taxonomic).
After the presentation of the dataset regarding the description of the vegetation, the next section describes the climatic and management variables used in the chapter.

B. Presentation of climatic and management variables

Nineteen variables were used to describe the climate and the management. The objective was to have the largest possible set of variables using the available data in the dataset. The variables were selected during a meeting of MULTISWARD experts. Data from farmer interviews was available on fertilization management and utilization of grasslands (number of cuts, number of animals). The information was partially incomplete and with varying levels of accuracy between the different datasets. Some corrections and aggregation of information were already done for the management data in the French national typology. Some corrections were also made for the Massif Vosgien dataset. In this case, some raw data was no longer available. In the Swiss Alps dataset, management data was not used prior to this study, and thus only raw data was available. This raw data was sometimes of poor quality. The climatic data were obtained afterwards using models or data from climatic stations.
Only climatic and management variables were used. However, they are not the only variables that affect the vegetation of grasslands. Soil characteristics also affect the vegetation. But soil variables were only recorded for the Swiss Alps dataset, so measured soil variables could not be used for the entire dataset. One alternative would have been to use soil maps like the European soil maps (Panagos, 2006; Panagos *et al.*, 2012). However, the spatial resolution was low. The quality of this map was tested on a set of grassland plots by comparing the measured variables and the variables on the map. The information was too poor at the plot scale (results not shown) to be used in this study. The second option would have been to use ecological indexes such as the Ellenberg indexes. The utilization of Ellenberg indexes as drivers of vegetation is problematic because Ellenberg indexes are calculated from the botanical composition. Thus, an utilization of the Ellenberg indexes would have induced a circular analysis (Zelený and Schaffers, 2012).
The grassland vegetation, especially the species richness, may also be influenced by elements of the landscape. The landscape may be described using remote sensing images with a high resolution, but the quantity of work needed to describe the landscape around our 439 plots was too longue to be realized during the PhD.
The following paragraphs present the different variables of climate and management with some description on the methods of their calculation, as well as their distribution in the dataset.

1. Altitude **(AL)**

This variable describes the average elevation of each plot in meter above sea level (continuous variables). The data were measured by GPS (Swiss Alps and French national typology) or calculated with an elevation model (Massif Vosgien). The altitude showed an almost uniform distribution with an average of 929m and a standard deviation of 566m. For the altitude, the dataset contained more grassland between 0 and 200m (66 plots) and between 1400 and 1600m (97 plots) that for the other categories of altitude (around 45 for the other categories).

2. Yearly rainfall **(YR)**

This variable describes the total annual amount of rainfall in mm. For the climatic variables, average climatic data was used (over at least one decade).The origin of the data differed between the different datasets. For the French national typology dataset, the closest monitoring station of Météo-France was used. When the closest station had a large altitude difference with the plot, another one further away but at a similar

altitude was chosen. For the Massif Vosgien dataset, climatic variables were obtained using a spatial model taking the altitude and the exposition into account. The model was fitted on Météo-France data. For the Swiss Alps dataset, climate data was obtained from a climatic model of MeteoSwiss. The model was fitted on MeteoSwiss data.

3. Rainfall during summer **(SR)**

This variable described the total annual amount of rainfall during the three summer months (June, July and August) in mm of rainfall. This variable may show potential drought during summer. The origin of the data was the same as for the yearly rainfall.

4. Temperature during the winter **(WT)**

This variable described the average temperature during the winter (from the 1st of October to the 31st of March) in C°. The origin of the data was the same as for the yearly rainfall.

5. Temperature during the vegetation period **(VT)**

This variable described the average temperature during the vegetation period (from 1st of April to 30th of September) in C°. The origin of the data was the same as for the yearly rainfall.

6. Potential grass production **(PR)**

This variable is an estimation of the potential grassland yield. The potential grass production was estimated from remote sensing images. Normalized difference vegetation index (NDVI) was computed from the red and infrared radiation from the MODIS images. The resolution of the MODIS images is of 250m x 250m. The NDVI were extracted from the MOD13Q1 and MYD13Q1 (the best image of 16 days period for the Terra and Aqua satellites). Information was extracted from the two years period around the period of the survey. NDVI signal was filtered and resampled to obtain monthly NDVI (le Maire *et al.*, 2011). On 217 grasslands where the grass production and the NDVI were recorded, a random forest (for a description of the analysis see Chapter 3 III) was used to explain the different monthly NDVI variables to the measured grass production. This random forest explained 33% of the variance of the measured grass production and was afterwards used to estimate potential grass production for all the plots. Even though the potential grass production was not really a climatic variable, we classified this variable with the climatic ones. Indeed, the potential grass production was strongly related to the climatic conditions (see the II C).The distribution of these climatic variables within the three datasets is presented in Figure.3.3

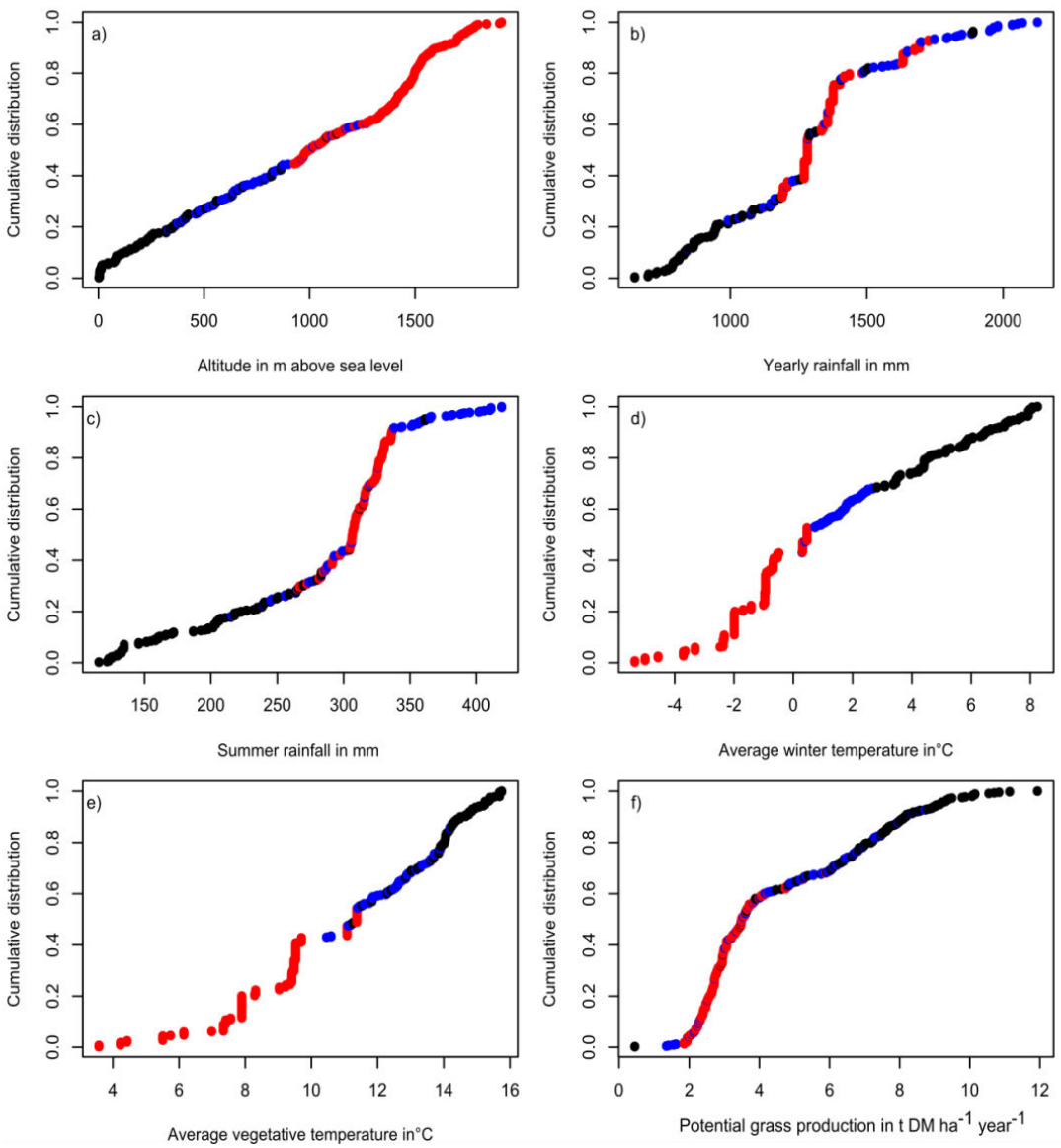


Figure.3.3: Distribution of the climatic variables. a) Altitude, b) Yearly rainfall c) Summer rainfall, d) Average winter temperature, e) Average vegetative temperature and f) Grass production. In black the French typology dataset, in blue the Massif Vosgien dataset and in red the Swiss Alps dataset.

7. Type of yearly use **(PCM)**

This variable assigned the type of grassland utilization to one of three categories: grazing only (P for pastures), cutting only (C), and a mixed utilization with both grazing and cutting (M).

Table.3.2: Proportion of the different type of yearly use in the different datasets in percentage

	Pasture	Cut	Mixture
All dataset(n=439)	0.28	0.31	0.41
French typology(n=140)	0.49	0.15	0.36
Swiss Alps(n=229)	0.10	0.47	0.43
Massif vosgien(n=70)	0.45	0.16	0.39

Grasslands with only a short pasture at the beginning of the season were classed as mixed utilization. Data originated from the farmer interviews.

8. Type of first use **(TFU)**

This categorical variable described the type of first use in two categories. P if the first use of the grassland is a pasture and C if the first use of the grassland is a cut. The data originated from the farmer interviews.

Table.3.3: Proportion of the type of first use in the different datasets in percentage

	P	C
All dataset(n=439)	0.36	0.64
French typology(n=140)	0.58	0.42
Swiss Alps(n=229)	0.19	0.81
Massif vosgien(n=70)	0.43	0.56

9. Number of cuts per year **(NCPY)**

This variable described the number of cuts per year. The data originated from the farmers interviews. Within the three datasets, no grasslands had more than 3 cuts per year.

Table.3.4 Proportion of plots with 0, 1, 2 or 3 cuts per year in the different datasets in percentage

	0	1	2	3
All dataset(n=439)	0.27	0.40	0.26	0.06
French typology(n=140)	0.49	0.32	0.12	0.06
Swiss Alps(n=229)	0.10	0.53	0.34	0.04
Massif vosgien(-n=70)	0.43	0.12	0.31	0.12

10. Stocking rate (days LU ha^{-1} $year^{-1}$) **(SRPY):**

This variable described the yearly stocking rate. The number of grazing animals is transformed in livestock units using the coefficient presented in the Table.3.5 as used by Michaud *et al*. (2012b).
We only used average coefficient per type of animals. We did not apply corrections regarding the breed or the milk productivity of the animals. The number of grazing animals was obtained from the farmer's interviews. For the Swiss data, the information was sometimes very poor and grazing only limited to autumn or spring pasture. This management is a typical management in mountain farms in Switzerland while climbing to the summer pastures animals stay a short time in the plots situated between the summer pasture and the farm buildings. Such plots might not be separated by fences; therefore management data may not be very accurate. We estimated this type of pasture to 35 days LU ha^{-1} based on an estimated available biomass of 500 kg DM ha^{-1} (based on the expertise of the Agroscope research team). The stocking rate of each different pasture event was summed at year level. The raw data originated from the farmer interviews.

Table.3.5: Livestock coefficients by type of animals used to transform number of animals into livestock units.

Type of animals	LU
suckler cows +calves	1
suckler cows	0.85
beef	1
calves	0.3
heifer 1 year	0.6
heifer 2 year	0.7
dairy cow	1
sheep	0.15
sheep + lambs	0.15
lambs	0.06

11. Date of first use **(DFU)**

This variable described the date of first use in Julian days. The date of the first use was obtained from farmer interviews. The accuracy of the answer to the question of the interview was sometime poor (only to the month). To keep the variable as a continuous variable and harmonize the information between the datasets, the less accurate data were transformed: when only the month of first use was available, the date of first use was approximated to the 15th of that month.

12. Sum of temperature at the first use **(SDFU)**

This variable described the sum of temperature at the first use of the grassland in d C°. In comparison with the date of first use, the sum of temperature at the first use takes into account the difference of climate especially during the winter. The temperature data presented above were used to calculate the sum of temperature at the date of the first use. The base of the sum of temperature was 0°C and the count started on the 1st of February (Theau and Zerourou, 2010).

13. Intensity of grass use index **(GU)**

Our dataset contains mown, mixed use and grazed grasslands. The number of cuts and the number of animals (or stocking rate) could not be compared or combined. We therefore needed a way to compare the intensity of defoliation between these different types of utilization. Different indexes exist in the literature to evaluate this intensity. Some indexes rely on a normalization of the number of cuts and stocking rate at regional scale (Herzog *et al.*, 2006) or are based on field measurements (Lienin and Kleyer, 2012). Field measurements were not feasible in our study and the normalization by region is problematic for regions with large differences in pedo-climatic conditions within a small spatial scale like in mountainous regions. The existing indexes were thus not adapted to our work and we developed a new one. **The method to calculate the intensity of grass use is presented in the Annex II**. The grass use index relied on two models linking stocking rate and percentage of defoliation: one for unproductive grasslands and one for productive grasslands. The separation between unproductive and productive grassland was based on the grass production estimated

from remote sensing images (see grass production description) with a threshold at 2.5 t DM ha^{-1} $year^{-1}$.

14. Relative grass use intensity index **(RGU)**

This variable is an index of grass use normalized by the climatic conditions. Indeed, a same management would not have the same effect in high productive grasslands than on low productive grasslands. The relative grass use index is obtained by dividing the grass use index (see variable 13) by the potential grass production (see variable 6).

15. Number of utilizations **(NU)**

The number of utilizations is the yearly sum of the number of cuts added to the number of pasture passages per plot. The number of utilizations can be used also as indicators of the intensity of use. It is also related to the frequency of use. The data was derived from the farmer interviews.

Table3.6: Proportion of plots with 1, 2, 3 or 4 and more utilization per year in the different datasets in percentage

	1	2	3	4≤
All dataset(n=439)	0.30	0.25	0.29	0.16
French typology(n=140)	0.21	0.25	0.23	0.31
Swiss Alps(n=229)	0.24	0.31	0.32	0.13
Massif vosgien(-n=70)	0.38	0.24	0.33	0.05

16. Disturbance index **(D)**

The disturbance index was obtained by the multiplication of the grass use index (GU) and the number of use (NU). This index assessed the frequency of use and the intensity of use. This equivalent index is already used in the literature (Lienin and Kleyer, 2012).

17. Nitrogen mineral fertilization **(NMF)**

This variable described the amount of mineral nitrogen fertilizer applied per area and expressed in kg N ha^{-1} $year^{-1}$. The data derived from the farmer interviews. The mineral inputs were null on 377 grasslands.

18. Nitrogen organic fertilization **(NOF)**

This variable described the amount of organic fertilizers applied per area and expressed in kg N ha^{-1} $year^{-1}$. The quantity of organic fertilization was transformed in a quantity of nitrogen (in kg) using the coefficients presented in table.3.7 as used by Michaud *et al.* (2012b). The organic inputs were null on 218 grasslands.

Table.3.7: Coefficients of quantity of nitrogen for the type of organic fertilization.

in t or m^3 ha^{-1}	Coefficient (kg N t^{-1} or kg N m^{-3})
solid manure	5
Compost	6.1
sheep solid manure	10.8
liquid manure	3
sheep compost	6.5

The quantity of organic fertilization derived from the farmer interviews.

19. Nitrogen total input **(NTOT)**

This variable describes the sum of all the nitrogen inputs made by the farmers (fertilization and nitrogen animals' returns). This variable was obtained by adding the mineral fertilization (see above) and organic fertilization (see above) and an estimation of the quantity of nitrogen in the animals' returns.
The estimation of nitrogen animals dejection per LU per days was based on the information provided in Bloor *et al.*(2012) and presented in the Table.3.8. The total nitrogen inputs were null on 78 grasslands.

Table.3.8: Coefficients of quantity of nitrogen for the animal dejection per LU per days

	N kg $days^{-1}LU^{-1}$ or animals
Cow	0.1218
Sheep(per animals)	0.0167
Sheep(per LU)	0.1111

The raw data (number of animals and fertilization quantity) originated from the interviews with the farmers.

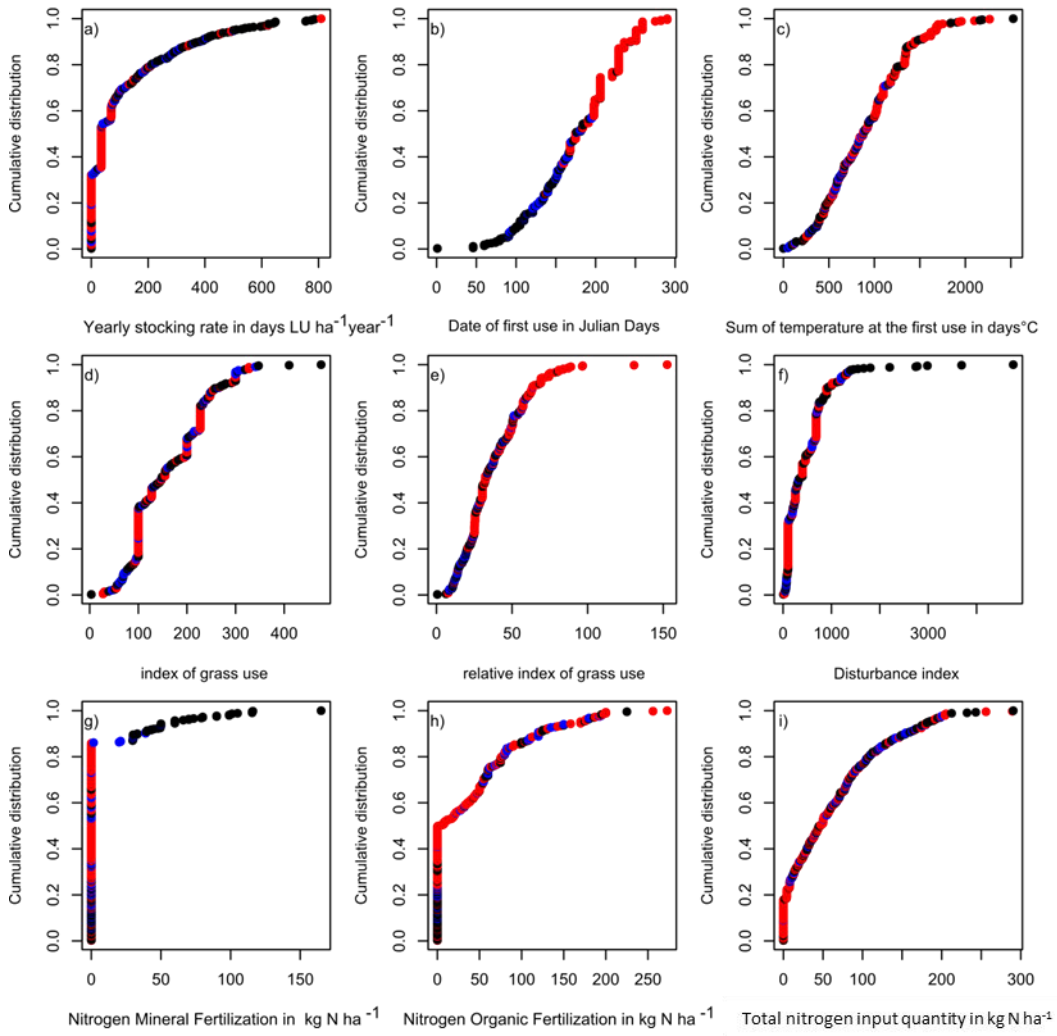


Figure.3.4: Cumulative distribution in the database of the management variables .a) stocking rate, b) date of first use c) sum of temperature at the first use, d) grass use index ,e) Relative grass use index f) disturbance index, g) Mineral nitrogen input , h) Organic nitrogen input and i) Total nitrogen input. In black the French typology dataset, in blue the Massif Vosgien dataset and in red the Swiss Alps dataset.

The distribution of stocking rate, date of first use, sum of temperature at the first use, grass use index, relative grass use index, disturbance index, mineral nitrogen input, organic nitrogen input and total nitrogen input in the database is presented in Figure.3.4.
Table.3.9 presents the average value, minimal value, maximal value and the standard deviation of agricultural management and climate variables.

Table.3.9: List of the different variables of management and climate used; for the continuous variables the mean and the standard deviation are presented. For the discrete variables, the numbers of plots on each category were reported.

	Description	mean	sd	min	max	Unit
PR	Grass production estimated by Remote sensing	4.59	2.39	0.45	11.93	$tDMha^{-1}$
VT	Temperature during the vegetative period (April-October)	11.06	2.87	3.58	15.75	C°
WT	Temperature during the winter (October-April)	1.32	3.36	-5.34	8.24	C°
YR	Sum of Yearly rainfall	1273.9	307.4	649.8	2126	mm
SR	Sum of summer rainfall (June, July and august)	282.40	67.0	115.6	419	mm
AL	Altitude	953.2	555.55	2	1910	m
NCPY	Number of cut per year	1.1	0.9	0	3	
SRPY	Yearly stocking rate per year	119	171.4	0	809	$dLUha^{-1}$
DFU	date of first use	175.8	52.86	1	290	Jd
SDFU	sum of temperature at the first date use	899.06	441.81	0	2526.1	dC°
GU	Grass use index	162.21	77.32	3.35	475.77	
NU	Number of use	2.41	1.46	1	11	
D	Disturbance index (GU*NU)	472.31	491.4	6.7	4758.7	
RGU	Relative grass use index (GU/PR)	37.64	20.31	0.61	152.6	
NMF	Mineral nitrogen quantity	8.07	23.07	0	165	$kgN\ ha^{-1}$
NOF	Organic nitrogen quantity	39.84	57.95	0	272.82	$kgN\ ha^{-1}$
NTOT	Total quantity of nitrogen input (NMF+ NOF+ animals dejection)	61.46	60.39	0	290.44	$kgN\ ha^{-1}$
	Discrete variables					
PCM	Pasture , Cut , mixed utilization	C:138	M:177	P:124		
TFU	Type of first use (Cut / pasture)	C:283	P:156			

C. Links between management and climatic variables

1. Materials and methods

We assessed the links between these different variables of management and climate in two different ways: one to assess the general links and the other was to regroup the variables.
We first used a principal component analysis (PCA) for the assessment of the general links between variables. The two discrete management variables (PCM and TFU) were added as additional qualitative variables. Some of the variables were transformed to normalize their distribution. Normal distributions simplify the use of PCA. The yearly stocking rate (SRPY), the sum of temperature at the first use (SDFU) and the 3 nitrogen variables (NMF, NOF, NTOT) were transformed using an ln(X+1) equation. The grass use index (GU), the relative grass use index (RGU), the number of utilization (NU) and the disturbance index (D) were transformed using an ln(X) function.
The PCA was performed using the FactoMineR packages on R software.
The relationships between these variables did not necessarily follow the same pattern; the classical correlation coefficient (Pearson) used in the PCA would not assess all the relationships accurately, and so the PCA did not accurately assess linear relationships. Indeed, the Pearson correlation only assesses linear relationships. We therefore used

the MIC correlation coefficient (Reshef *et al.*, 2011): this coefficient is able to detect any form of relation between two variables (Speed, 2011). The MIC coefficient was computed for each couple of variables. In order to graphically represent these MIC correlations, we calculated 1-MIC coefficient. The calculation gives information about the dissimilarity between two variables. Indeed, variables with a high correlation have a small dissimilarity of 1-MIC. These dissimilarities (1-MIC) were then projected on a dendrogram using an agglomerative nesting using the Ward methods (Kaufman and Rousseeuw, 1990). These correlation analyses were used to see the links between continuous variables. For the discrete variables, we performed a Kruskal-Wallis test to evaluate the correlation of the discrete variables and the continuous variables.

2. Results and discussion

The first dimension of the PCA (38.17%) was positively linked to the temperature, the stocking rate, the nitrogen mineral fertilization and negatively correlated to the altitude, the rainfall, the date of first use and the sum of temperature at the first use (Figure.3.5a). The second dimension (24.06%) was positively correlated with the relative grass use, the grass use, the number of cuts per year, the nitrogen organic fertilization, the disturbance, the number of uses and the total nitrogen inputs. At higher altitude, more grasslands were mown at the first utilization and the type of use “cutting only” was more frequent than at lower altitude where grazing for the first utilization and the type of use “grazing only” were more frequent. The type “mixed utilization" was the most intensive one (results not shown).

The different climatic variables were strongly correlated to each other (Figure.3.5) either with the PCA or the MIC correlation. The large altitude gradient seemed to be the main driver of the temperature gradient in our dataset. Rainfall was also related to the altitude. This link may partly be due to abundant snowfalls at higher altitude.

Most of the management variables were not related to the climate with the exception of the two variables related to the timing of use (DFU and SDFU). Indeed, the date of first use was strongly defined by the climate. Even when the date of first use was adjusted to the climatic difference (sum of temperature at the first use), the timing of the first use was still correlated with the climate. The type of use and first use were also related to the climate. Two main explanations can be found for this pattern: first, at high altitudes, the winter season lasts longer so the quantity of conserved forage per livestock unit has to be larger. Conversely, grazing season lasts longer in the lowlands. Secondly, the plots in mountain areas, especially the ones from the Swiss dataset, were part of farms which also had summer pastures. Summer pastures were not surveyed in the studies from which the data originated. In summer pastures, the grazing pressure is very heterogeneous within a single pasture and its effects on the vegetation can hardly be studied using only management criteria at the summer pasture level (Homburger *et al.*, 2013). Indeed, during summer, the animals graze mainly the summer pastures. In this period the others plots are less grazed and so the spare grass is harvested to created forage stocks. The proportion of mown plots is also higher. For the other management variables, especially the aggregated intensity variables (NTOT, GU and RGU), the links with management were very weak. It would have been logical to find, on average, a more intensive management at low altitude (good climatic conditions).

However, in the lowlands, some extensive plots also exist. This result could be explained by the obligation of keeping ecological compensation zones and also by the fact that management is decided at the farm level. Indeed, in each farm, some plots were more intensively managed, and others more extensively (Roche *et al.*, 2010). The management was also very variable within each altitude group.

Moreover, in the French typology dataset that included lowland areas, the number of utilizations did not exceed four utilizations per year, which showed that very intensive grassland management systems (6 to 7 utilizations per year) were not represented in the lowlands.

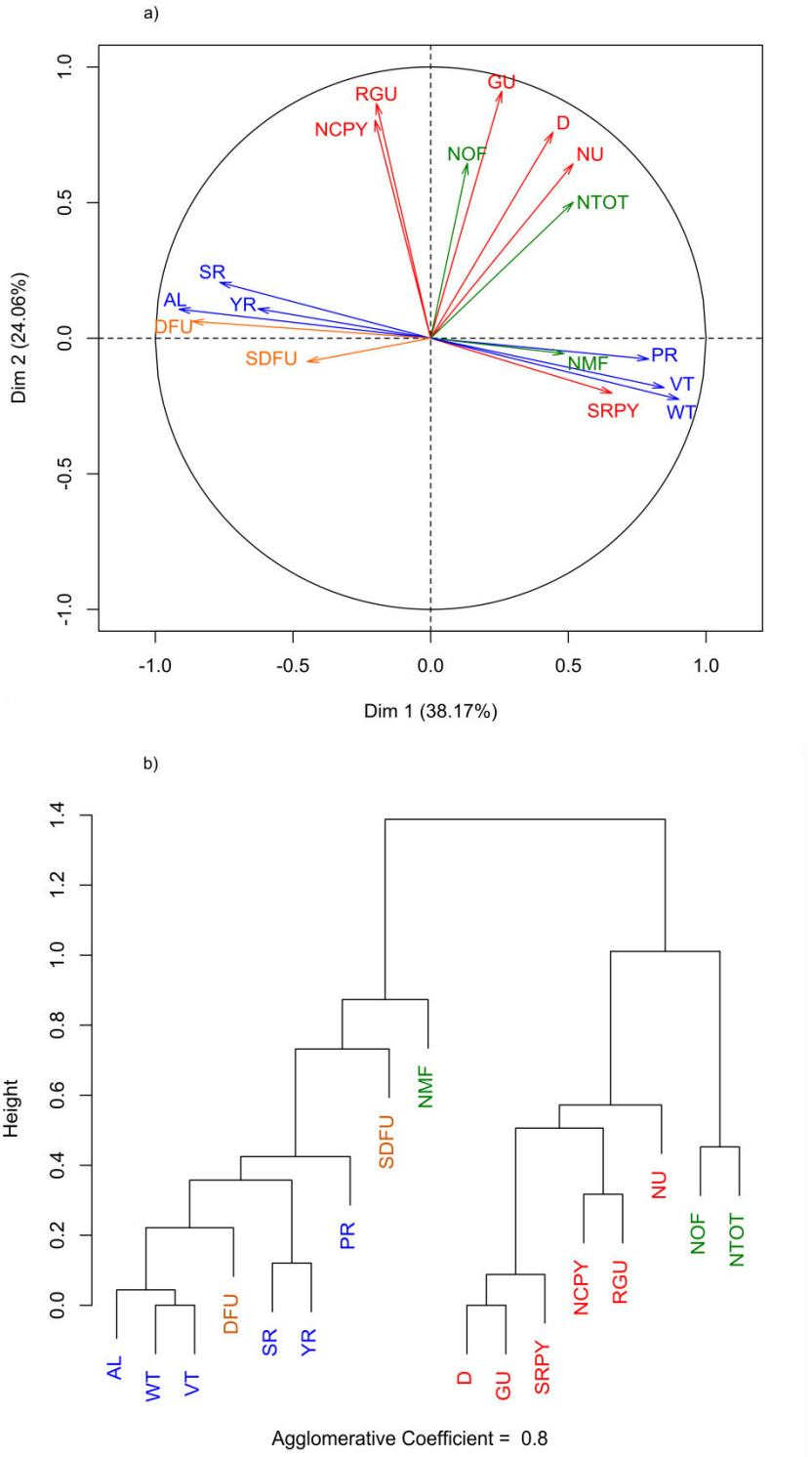


Figure.3.5:a) Principal component analysis on the different management and climatic variables. See the abbreviations in Table 3.12. The functional diversity criteria were added as supplementary variables. b)Dendrogram issue of agglomerative nesting using the Ward method on the MIC dissimilarity (1-MIC coefficient) for the management and the climatic variables (explanations of the abbreviations of the variables in Table.3.12). In red the grass use variables, in green the variables related to the fertilization, in orange the variables related to the timing of use and in blue the variables related to the climate.

The two analyses were complementary. The PCA was very useful to see the general trends between the variables and thus to regroup the variables.

For the rest of the chapter, we made four groups of variables related to each other: the climatic variables (AL, WT, VT, SR, YR, PR), the timing of use variables (DFU, SDFU), the nitrogen fertilization variables (NTOT, NOF, NMF) and the disturbance variables (D, NCPY, NU, SRPY, GU, RGU).

III. Choice of Statistical Analyses

A large set of statistical methods can be used to test the effect of management and climate on biodiversity. We had to choose between several analysis that were able to answer our questions (i.e. the evaluation of the effect of management and climate on functional diversity following general trends or/and conditional effects and estimation of functional diversity) but also able to deal with the high number of explanatory variables. We reviewed and evaluated different types of analysis methods. This evaluation is summarized using a score for four different capacities of the method.
We assessed the ability of each method to evaluate general trends (1) or/and conditional effects (2). We also evaluated the ability of the method to take into account the interrelations in the analysis (3). Finally, the capacity of the different analyses to predict functional diversity criteria was judged (4). This capacity was evaluated based on the capacity of the analysis to avoid overfitting.
For the different capacities, we propose a score with four modalities: + if we judged that the method was good; = if we judged that the method was neither good nor poor; - for a poor capacity and / if the method is unsuitable (Table.3.10). The scores were attributed based on our expertise after discussions with Dr Jean Villerd and Dr Knut Hovstad.
ANOVA/linear model families are generally used to test effects but can also be used for prediction or estimation. Multilinear models (MLM) are useful for assessing general trends. The **ANOVA** and **ANCOVA** are more suitable for dealing with conditional effects on the differences of linear model. The main limitations of these analyses are the conditions of application. Covariance between the explanatory variables can be problematic for the interpretation of results. One option is to make a preliminary analysis to regroup some of the explanatory variables and use the outputs of these analyses as input of the model (cluster analysis for the ANOVA, PCA for the multi-linear model; MLMPCA). **Stepwise** variable selection can be used to simplify the complex multi-linear model and limit over fitting. In a stepwise variable selection, a selection of the different explanatory variables is made in order to propose the most parsimonious model by iteratively removing each variable and using AIC criteria for estimating the compromise between fitting and parsimony.
Structural equation modeling (SEM) is a statistical technique for testing and estimating causal relations using a combination of statistical data and qualitative causal assumption. **Structural equation modeling** relies on a set of models where the relationships between explanatory variables are included (Shipley, 2002). Latent variables can also be implemented in the structural equation modeling (Rosseel, 2012). A latent variable is a variable not observed but inferred from other observed variables. A latent variable is a way to include important concepts.
Canonical correlation analysis (CCA) analyzes the correlations between two different sets of variables (management and climate one way, biodiversity the other way, for example).
Partition of variance assesses the percentage of variance explained by different sets of variables (management, climate for example) and the variance explained jointly by different sets of variables(Borcard *et al.*, 1992; Legendre and Legendre, 2012).
Regression tree analyses originates from machine learning (Breiman *et al.*, 1984 ; Breiman, 2001). In the **regression tree**, the response variable is split into groups according to one of the explanatory variables, in order to minimize the variances within-group and maximize the difference in between-group variance. The process is then repeated within each group iteratively. The **random forest** or **Breiman forest** is a set of regression trees. For each tree, one part of the data is kept to evaluate the quality of

the tree. Furthermore, in each tree only one part of the explanatory variable is used. The importance of one variable is evaluated based on the difference between the tree where the variable is inputted and the trees where the variable is not inputted.

Table.3.10: Scoring of the different analysis methods regarding their capacity to assess conditional effects, general trends, relationships between variables and prediction of the functional diversity criteria(+ stands for “good”, = stand for “neither good nor poor”, - stand for “poor” and / stands for “unsuitable)

Analysis	conditional effects	General trends	Relationships between explanatory variables	Prediction
ANOVA	=	=	-	-
ANOVA on cluster	=	=	=	-
MLM	/	-	-	=
MLM PCA	/	=	=	-
ANCOVA	=	=	=	=
STEPWISE	-	+	=	+
SEM	/	+	+	=
Regression trees	+	-	-	+
Random Forest	+	-	+	+
CCA	/	=	+	/
Variance partitioning	/	-	+	/

No method had a good score for all the different categories. A combination of analysis was necessary to answer to all our questions.
In the literature, the effect of management and climate are generally assessed using simple methods (ANOVA or linear model). Regarding our large number of variables, these analyses were not adapted.
For the evaluation of general trends, we used the variance partition and the structural equation model.
We chose to assess the conditional effect using a combination of regression tree and random forest.
The variance partition and the structural equation model have already been used in the literature to test the effect of management and climate on biodiversity (Dainese *et al.*, 2012) for the variance partition and for the structural equation model (Douma *et al.*, 2012). We did not find studies using the random forest or the regression trees.
The analyses were also performed on a dataset resampled from the entire dataset with better distribution of the altitude.

IV. Assessments of the general trends between management, climate and diversity of grasslands,

A. Objective

The objective of this section is to evaluate the general trends among management, climate and functional diversity of permanent grasslands. These trends could be assessed using linear models. However, the large covariance between the different variables of climate and management could hide some effects or induce false effects. We first had to evaluate the importance of this covariance in the explanation of functional diversity variability. We made a variance partition to evaluate the importance of the covariance between the different variables on the explanation of functional diversity.

Secondly, we tested different models taking the relationship between the different variables (management, climate, functional diversity) into account: structural equation models (SEM). One interest of SEM is to be able to separate the direct and indirect effects. For example, if variable A affects variable B, this effect is indirect if the variable A affects another variable (C) and this variable C, in turn, affects variable B. The effect is direct if the effect of the variable A affects variable B without any intermediate variables. Structural equation models may be used to explore the relationships between variables in a dataset or to test different hypotheses of correlations. In exploring relationships between variables, the dataset is explored to construct the model of path relationships. However, a model constructed this way is representative of the dataset and a validation using an independent dataset is necessary. The other possibility is to use the SEM to test different hypotheses of relationships between the different variables. We chose the second option, to test some hypotheses of correlations between variables. Indeed, our objective was to be able to estimate functional diversity from management and climate.

B. Material and methods

In order to make first assessments of the general trends, we projected the different functional diversity criteria on the PCA in Figure 3.5A as supplementary variables.
The data used was the transformed data used for the PCA. Some of the variables were transformed to normalize their distribution (see section C.1.).

1. Partition of variances

To quantify the effect of the interrelations between the climatic and management variables on the functional diversity criteria, we used a partition of variance analysis (Borcard *et al.*, 1992; Legendre and Legendre, 2012). Different grouping of variables were possible. We decided to separate the explanatory variables in four groups: disturbance (grass use variables), fertilization, timing of use and climate (temperature, altitude, rainfall). In this section we discuss only the functional diversity criteria with more than 30% of variance explained and analyzed with structural equation modeling.

2. Structural equation modeling

We tested five different models on the functional diversity criteria. For each model, we proposed different causal relations between different types of variables: climate and management.
In the first model (M1), the main hypothesis tested is that the climate influences the basic management such as the number of cuts, stocking rate and organic and mineral fertilization.
We used eight variables: altitude, winter temperature, number of cuts, stocking rate, grass use index, nitrogen organic fertilization, total nitrogen inputs and the date of first use. We tested different causal relationships between these variables:

- The altitude affects winter temperature (lower temperature in altitude).
- The altitude affects the number of cuts per year. This is based on the assumption that in the highest altitudes, the need for stocking forage is higher due to the longer winter.
- Winter temperature influences the date of first use (a warmer winter induces an earlier use).
- The winter temperature also influences the yearly stocking rate, as a warmer winter allows a longer grazing season.
- The number of cuts per year and the yearly stocking rate were not directly influenced by the same variables.
- We assumed that the grazing was more affected by winter temperature. Grazing is possible only if the temperature is not too cold.
- The date of first use was also defined from the winter temperature.

In this model, the functional diversity criterion was related to the altitude, the winter temperature, the date of first use, the grass use and the nitrogen total input. The structure of the model is presented in Figure 3.6a

The second model (M2) was very similar to the model M1, however the effect of climate was not directly linked to basic management variables (number of cuts, stocking rate…) but rather to management intensity (i.e. total nitrogen inputs, grass use index…). In this model, we changed the causal relationships of the altitude on management. The altitude affected the intensity of use and total nitrogen input, and not the yearly stocking rate and the number of cuts per year. In order to simplify the model we deleted the links between nitrogen organic fertilization and number of cuts.

The third model (M3) was an extension of the model M1. We added a qualitative variable into the model: the type of yearly use (PCM). The main hypothesis is that the yearly type of use is a choice of farmers (choice between pure cuts, pure pastures and mixed) and that this choice determines the number of cuts and the stocking rate together with the altitude and the winter temperature. A link between the number of cuts and nitrogen fertilization was removed from the model.

One of the main advantages of structural equation models is the possibility to include latent variables in the model. The latent variables are unmeasured variables that can be included into the model to describe a concept. The value of the latent variable is fitted to the variables most closely related to the latent variables. We used two latent variables in two of our models: one variable to describe the climatic conditions and one to describe the intensity of management. The latent variables were the same in the two models. A climatic latent variable was defined from the yearly rainfall, winter temperature and altitude. An intensity of management latent variable was defined from the disturbance and nitrogen total inputs. The latent variables were calculated to have the best aggregation of the different management and climatic variables, and to propose the best explanation of the biodiversity.

The climatic latent variable had an influence on the management intensity latent variable. In the first model with latent variables (M4), the functional diversity criterion was only affected by the two latent variables. In the second one (M5), the date of first use was inserted in the model. The date of first use was influenced by the climatic latent variables and the functional diversity criteria.

Our objective was to implement these five models for different levels of functional diversity (the ones with more than 30% of variance explained in the variance partition) using the laavan package in the R software (Rosseel, 2012). We then compare the five different models in order to find the best model and to conclude on the quality of the fit based on the thresholds proposed in the literature (Hooper *et al.*, 2008). Different types of fit indicators can assess the quality of fit for structural equation models (Hooper *et al.*, 2008). We used a set of indicators: the chi-square, the root mean square error of approximation (RMSEA), the comparative fit index (CFI) and the Akaike Information Criterion (AIC). The different indicators were used to select the best model between the 3 models without latent variables (M1, M2 and M3) and the best one with latent variables (M4 and M5). The indicators were also used to assess the relevance of SEMs for the estimation of functional diversity criteria.

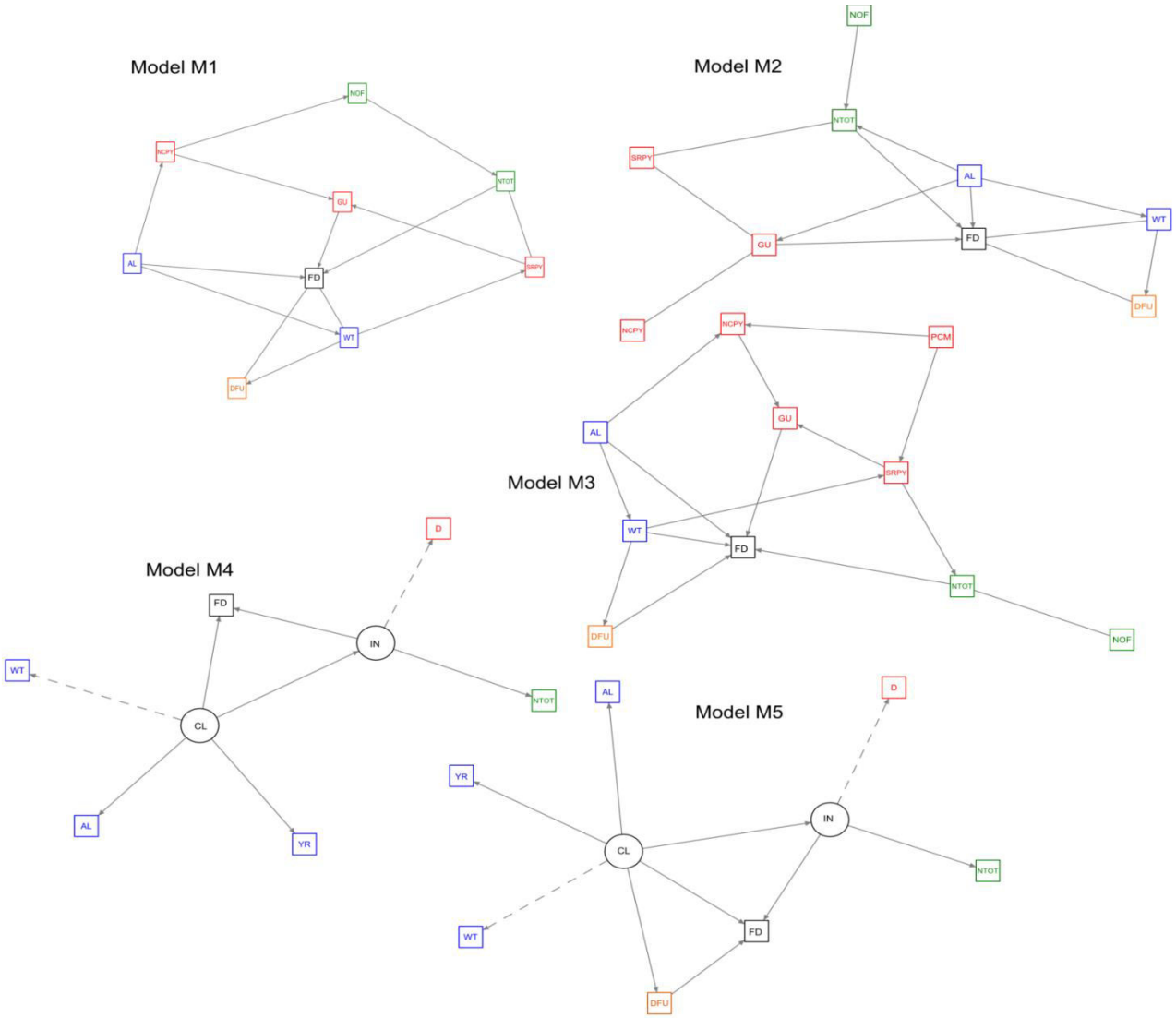


Figure3. 6: Different structural equation model tested. In red the grass use variables, in green the variables related to the fertilization, in orange the variables related to the timing of use and in orange

C. Results

On the PCA with a projection of the functional diversity criteria (Figure 3.7), only four of the functional diversity criteria were correlated to the different axes (number of species, functional identity of SLA, LNC and OFL). These criteria were mostly correlated with the first dimension of the PCA (**positively for CWMSLA and CWMLNC, negatively for the number of plant species and CWMOFL**).

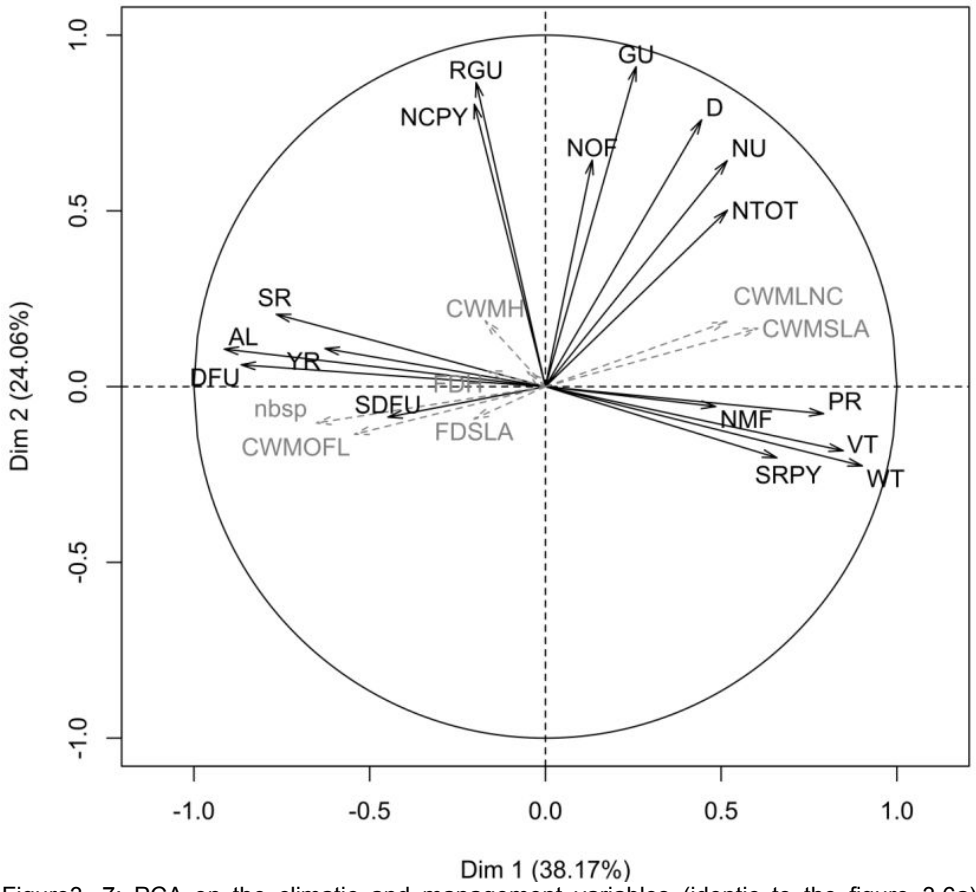


Figure3. 7: PCA on the climatic and management variables (identic to the figure 3.6a) with projection of functional diversity criteria as supplementary variable (in grey).

1. Partition of variances

The residuals (i.e. variances not explained by the model) were above 80% for three of the functional diversity criteria (CWMH and the two functional dispersion criteria). For the remaining criteria, 48% of the variance was not explained by the variance partition for the number of plant species, 56% for community weighted mean of SLA, 66% for the community weighted mean of LNC and 64% for the community weighted mean (Table.3.11). For the rest of the study, we only analyzed and discuss these last four criteria.

For these criteria, most of the explained variance is due to the combination of the four sets of variables: climatic (C), timing (T), nitrogen fertilization (N) and disturbance (D). 22% of the variance for the NBSP (number of plant species) was explained by the four sets of variables, 21% for CWMSLA, 12% for CWMLNC and 13 % for the CWMOFL.

The second most important combination of sets differed between the four functional diversity criteria. The effect of only climatic variables were the second most explanatory variable for the number of species (16% of the variance) and for the CWMLNC (6%).

Table.3.11: Results of the partition of variances for the CWMSLA, CWMLNC, CWMOFL and number of plant species criteria; C for the climatic variable (AL,WT,VT,YR,SR), T for the timing variables (SFU,SDFU), D for the disturbance variables (RGU,D,NCPY,GU,SRPY,NU) and N for the nitrogen input variables (NTOT,NOF,NMF).

	NSP	CWMSLA	CWMLNC	CWMOFL
C	16%	4%	6%	9%
T	1%	0%	0%	0%
N	2%	2%	1%	3%
D	2%	2%	1%	5%
CT	0%	2%	2%	1%
TN	0%	0%	0%	0%
CN	0%	0%	0%	0%
CD	1%	0%	2%	0%
TD	0%	0%	0%	0%
TN	4%	5%	1%	4%
CTD	6%	4%	10%	5%
CTN	2%	2%	0%	0%
TND	0%	1%	0%	1%
CND	0%	0%	0%	0%
CTND	22%	21%	12%	13%
Residuals	48%	56%	66%	64%

For the CWMSLA, the second most important combination was timing and fertilization (5%). For the CWMOFL, the second most important combination was timing, disturbance and fertilization (9%).

2. Structural equation models

For the four selected functional diversity criteria, the best model regarding the different indicators was M4 (Figure.3.6). Among the three models without latent variables (M1, M2, M3), the model M2 was the most accurate (Figure.3.6). The results for M2 and M4 are presented in Table 3.10.

For M2, the standard estimates of the relationships between management and climatic variables were identical for the four different criteria. Some effects were very strong (-0.91 for the effect of altitude on winter temperature); others were weak (-0.05 for the effect of altitude on grass use index).The R^2 for the functional diversity criteria were of 0.48 for the number of species; 0.43 for the CWMSLA, 0.32 for the CWMOFL and 0.31 for the CWMLNC.

In M2, the effect of the altitude on the functional diversity criteria was stronger than the effect of winter temperature for the specific leaf area (-0.23 for altitude versus 0.14 for winter temperature) and the onset of flowering (0.33 versus -0.15) at the opposite of the number of species (0.13 versus -0.47) and the leaf nitrogen content (0.01 versus 0.36). Between the nitrogen inputs and the defoliation intensity (grass use index), the nitrogen input had a stronger causal effect for the number of species (-0.21 versus -0.14) and for the CWMSLA (0.27 versus 0.14) at the opposite of the CWMOFL (-0.10 versus-0.13) and CWMLNC (0.16 versus 0.23).

For M4, the model with the best performance, the climatic latent variable was strongly related to the winter temperature and the altitude, and less influenced by the yearly rainfall.

The management intensity was more influenced by the nitrogen total inputs than by the disturbance. The links between the two latent variables were around 0.50 for all the functional diversity criteria. The climatic latent variable had a larger effect than the management intensity on the number of species (-0.52 versus -0.27) and on the community mean value of onset of flowering (-0.46 versus -0.17) than on the specific leaf area (0.38 versus 0.40) and the leaf nitrogen content (0.30 versus 0.35).

D. Discussion

General trends between climate, management and functional diversity criteria can be observed in our dataset. **The number of species and the onset of flowering increased with altitude (and decreased with temperature)**. **The specific leaf area** and **leaf nitrogen content decreased with altitude.**

Management also had an effect on functional diversity. **The intensity of defoliation** and **nitrogen inputs** had a **positive effect** on the community weighted mean value of the **specific leaf area and leaf nitrogen content** and a **negative effect** on the **onset of flowering and the number of plant species**. The covariance between the management and climate was not so strong. However, this interrelation had a strong effect on the functional diversity criteria. Indeed, the covariance of three types of management variables and climate was the most important combination in the partition of variance (Table 3.11).

In other studies that used a partition of variance to separate the effect of different factors on plant biodiversity, the percentage of variances explained by the covariance between the different sets of factors (management, climate, soil, landscape) was lower than in our results (Dainese *et al.*, 2012; Chanséaume *et al.*, 2013). This high percentage of variances due to the covariance could have been due to the presence of few management variables strongly correlated to the climatic variables. Indeed, the date of first use for the timing category, the nitrogen mineral fertilization for the fertilization category and the yearly stocking rate for the disturbance category were correlated to the climatic variables. Without these three variables, the percentage of the combination of the four types of variables decreased (16% instead of 22% for the number of species, 16% instead of 21% for the specific leaf area and 11% instead of 13% for the onset of flowering). The combination of the four types was still the most important combination.

This important effect of the covariance of climate and management on functional diversity criteria shows the interest of developing a structural equation model including the effects between climate and the different types of management variables. However, none of the different hypothesis we proposed produced a good model fit. Some links inputted into the models were obviously not strong and thus did not induce a good fit of the models. Indeed, the fit quality of a structural model depends on all the causal relationships implemented.

One option could be to delete these links and try to obtain the best model fitting this data by exploration. Nevertheless, the explorative use of structural equation models has to be validated on other datasets if the objective is to use it for prediction/estimation. We chose not to use the structural equation modeling for exploration, but only to test hypothesis.

The low quality of the structural equation models prevented their use to estimate functional diversity.

Using M4 with the latent variables, we can evaluate the relative strength of the climate and the management intensity on functional diversity criteria. For the onset of flowering and the number of plant species, the climatic effect was stronger than the effects of management intensity. For the specific leaf area and the leaf nitrogen content, the effects of climate and management intensity had the same strength (Table.3.12).

Table.3.12: Standard estimates of the model 2 and model 4 for the four diversity criteria. For the abbreviation see Table 3.9 .

			nbsp	CWMSLA	CWMOFL	CWMLNC
M2	R^2		0.48	0.43	0.32	0.31
	AL	WT	-0.91	-0.91	-0.91	-0.91
	WT	DFU	-0.74	-0.74	-0.74	-0.74
	AL	NTOT	-0.27	-0.27	-0.27	-0.27
	AL	GU	-0.05	-0.05	-0.05	-0.05
	NOF	NTOT	0.66	0.66	0.66	0.66
	SRPY	NTOT	0.43	0.43	0.43	0.43
	SRPY	GU	0.43	0.43	0.43	0.43
	NCPY	GU	0.93	0.93	0.93	0.93
	AL		0.13	-0.23	0.33	0.01
	GU		-0.14	0.14	-0.13	0.23
	WT	FD	-0.47	0.14	-0.15	0.36
	DFU		-0.08	-0.09	0.01	-0.04
	NTOT		-0.21	0.27	-0.10	0.16
M4	R^2		0.48	0.46	0.32	0.32
	WT		0.95	0.93	0.92	0.94
	AL	CL	-0.95	-0.98	-0.98	-0.96
	YR		-0.56	-0.54	-0.53	-0.55
	D		0.57	0.56	0.54	0.60
	NTOT	IN	0.89	0.91	0.94	0.85
	CL		0.49	0.51	0.50	0.51
	CL	FD	-0.52	0.38	-0.46	0.30
	IN		-0.27	0.40	-0.17	0.35

The strong effect of the climate on the onset of flowering is quite logical considering the very large gradient of climatic conditions in our dataset.

Among the climatic effects, the effects of altitude on the onset of flowering appeared to be more important than the effect of winter temperature (model 2; Figure.3.6). This fact is quite surprising. Indeed, the difference of onset of flowering with the altitude is generally explained by the difference of temperature. The average temperature of the winter may not be a good indicator of the effect of the temperature on the onset of flowering. Perhaps the date, when the frost stops (beginning of the vegetation period), would be a better indicator.

For the effect of management intensity on the onset of flowering, the defoliation intensity seemed to be more important than the early use of utilization and the nitrogen fertilization inputs. The effect of defoliation is more direct than the effect of fertility. Indeed, the defoliation (by grazing or cuts) may eliminate the species with a late flowering and therefore favor species with an earlier flowering (Fayolle, 2008; Lavorel *et al.*, 2011). The effects of nitrogen fertility (inputs) on the flowering date may be mainly indirect. Nitrogen fertility influences the functional strategy of the species, which is correlated with the flowering cycle (Navas *et al.*, 2010).

For the community weighted mean value of leaf nitrogen content (CWMLNC), the climatic effect was mostly due to the winter temperature (model M4). The leaf nitrogen content was related to the photosynthetic activity. The photosynthesis was positively influenced by the temperature and so it was quite logical to find an effect of temperature on leaf nitrogen content (Wright *et al.*, 2004). The effects of management on leaf nitrogen content, the

nitrogen input effect was almost equally as important than the effect of the grass use index. The effect of nitrogen inputs and nitrogen availability on nitrogen leaf content was expected (more nitrogen in the soil means more nitrogen available for the plant). The effect of disturbance is probably due to the influence of defoliation on the tradeoff between acquisition conservation of nutriments (Fayolle, 2008; Lavorel *et al.*, 2011). It could also be due to the correlation between nitrogen inputs and intensity of disturbance.
For the SLA, the effects were similar to the results of leaf nitrogen content, except that the effect of climate was more due to the altitude than to the winter temperature. It is difficult to explain these differences: these two traits are generally driven by the same factors and their correlation suggests a similar answer to the climate variables.
For the number of species, the model M2 showed that the most important management variables were the nitrogen inputs and the temperature for the climatic variables. The temperature and nitrogen availability are important factors that influence the growth of the plants. Cold temperature or a limitation in nutriments is an example of environmental stresses

E. Conclusions

We **observed general trends between management, climate and functional diversity** on a large scale. However, the strong covariance between the management and climate variables complicated the understanding of these trends and so the estimation of the plant functional diversity from management and climate variables. Structural equation models seemed to be a good option to take this strong covariance into account and propose causal effects between these variables. However, none of the different hypotheses we tested led to well fitted structural equation models. One possible explanation of the poor fit of structural equation modeling is that the relations between management, climate and functional diversity **cannot be assessed only as general trends**. Taking conditional effects into account could improve the quality of the estimation of functional diversity. The following section presents a study of the conditional effects of management and climate on grassland plant diversity.

V. Conditional effect assessments: Estimating functional diversity of grasslands from agricultural management and climatic conditions

A. Objective

The following part presents the assessments of the conditional effects of management and climate on functional diversity criteria. These analyses were based on a random forest and regression tree analyses.

B. Materials and methods

We analyzed the conditional effects of the different management and climate variables on functional diversity and the number of plant species using a regression tree approach (Breiman *et al.*, 1984 ; Breiman, 2001). In the **regression tree**, response variable is split into group regarding one of the explanatory variables in order to minimize the variances within-group and maximize the difference between-group variance. The process is then repeated within each group iteratively. The random forest or Breiman forest is a set of regression trees. For each tree in the random forest, a random part of the data is kept to evaluate the quality of the tree constructed on the other part (cross validation). Furthermore, only some of the explanatory variables are used in each tree to evaluate the importance of each explanatory variable. The importance of each variable is evaluated

from the difference of quality between the trees where the variable is inputted and the trees where the variable is not inputted.
For each functional diversity criterion, we made a random forest using all the management and climate variables. 30% of the data were randomly kept for the validation. We also included the origin of the data (Swiss alps, French national typology and Massif vosgien). 2000 regressions trees were computed with each time only 6 of the 20 variables. The percentage of variance explained by the random forest was used to evaluate the quality of the analysis. The importance of each variable was assessed by the mean decrease in accuracy when the variable was not used.
The random forest was computed using the randomForest function with R software (Liaw and Wiener, 2002).
In a second step, we built a regression tree. We only made the regression trees for the functional diversity criteria with more than 40% of variance explained by the random forest. The regression trees were built using only a selection of the climatic and management variables. The selection of the variables inputted in the regression tree was based on the results of the random forest, the MIC dendrogram and the PCA on the management and climate variables (see figure 3.5). The results of the random forest were used to select only the most important variables and those of the MIC and PCA analyses to eliminate some variables closely related to each other.
Regression trees were made using the rpart function with R software (Therneau *et al.*, 2012). We limited the minimal number of grasslands at the end of the regression tree (the categories created by the regression tree) to 22 (5% of the dataset). This limitation was defined to avoid some separations only due to few individuals.
We made an ANOVA followed by a post hoc TukeyHSD to test the difference in term of functional diversity criteria between the categories created by the regression tree. We also made an ANOVA to test the difference in term of management and climate between the categories created by the regression trees.
Inside each categories of the regression tree, we afterwards made linear regressions between the functional diversity variables and the different variables of management and climate to assess the eventual residuals effect of management/climate variables.

C. Results

1. Random forests

Four functional diversity criteria had more than 40 % of variance explained by the random forest (Figure.3.8): the number of plant species (61.57%), the community mean value of the specific leaf area (46.94%), the community mean value of the leaf nitrogen content (40.8%) and the community mean value of the onset of flowering (40.76%). These four diversity criteria were therefore further analyzed. The other variables had a percentage of variance explained below 25%: the community mean value of vegetative height (20.1%) the functional dispersion of SLA (8.1%) and the functional dispersion of H (8.1%).
The climatic variables generally influenced the analysis more than management variables. The altitude was the most important variable for almost all of the four functional diversity criteria. For the CWMLNC the altitude was the second most important variable but with almost the same mean decrease in accuracy as the winter temperature.

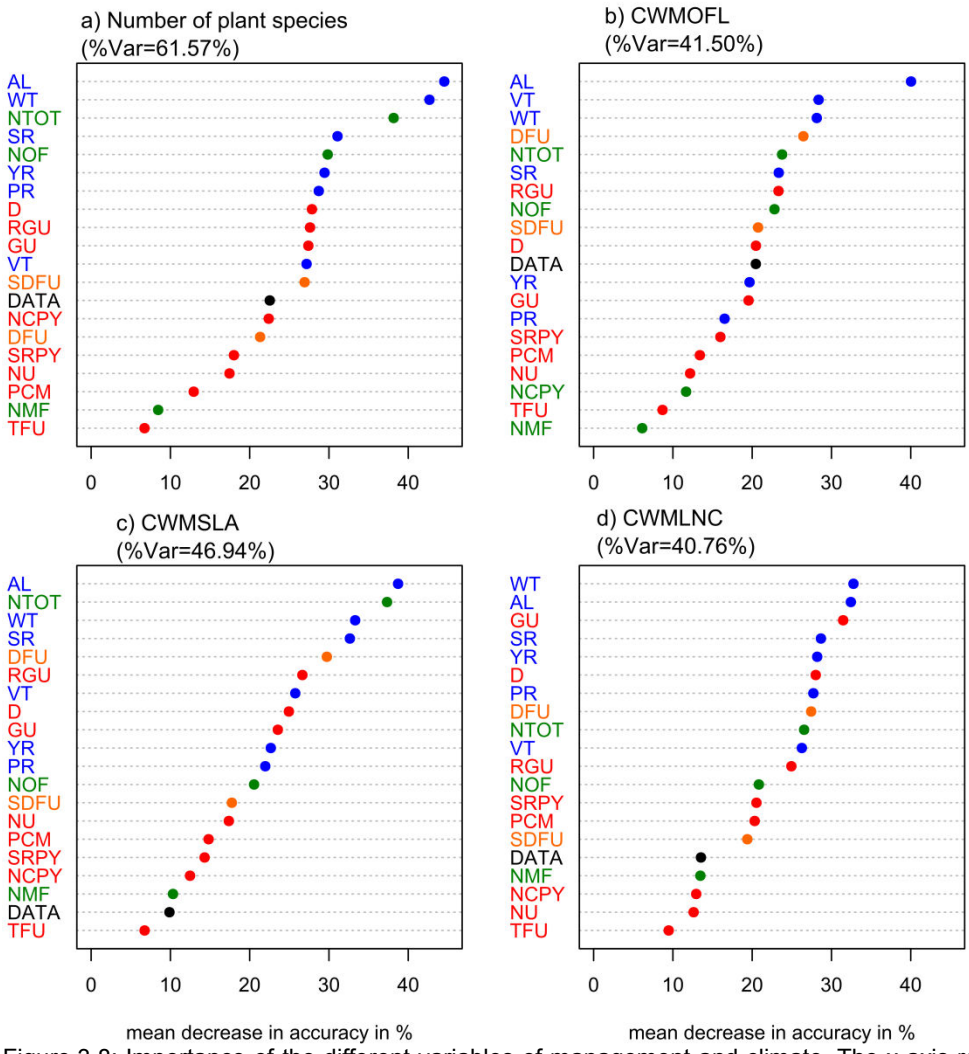


Figure.3.8: Importance of the different variables of management and climate. The x axis represents the mean decrease in accuracy of the cross validation of the regression tree in the randomForest. The abbreviation of the different variables of management and climate are presented in the Table 3.9. In red the grass use variables, in green the variables related to the fertilization, in orange the variables related to the timing of use and in orange. The importance of the variables: a) for the number of species, b) for the community weighted mean value of the flowering date, c) for the community weighted mean value of the specific leaf area d) for the community weighted mean value of the leaf nitrogen content.

The total quantity of nitrogen inputs (NTOT) was the most important of the management variables for the number of plant species, the CWMOFL and the CWMSLA. The grass use index was the most important variable of management for the CWMLNC. The aggregated indexes of management like the grass use index and the total nitrogen quantity were generally more important that the simple variables like the number of cuts and the mineral nitrogen content. The type of first use (TFU) and the type of yearly use (PCM) were not very important. The dataset of origin had not a lot of importance especially for leaf nitrogen content and the specific leaf area (Figure.3.8cd).

2. Regression trees

a. Number of plant species

The regression tree on the number of species computed with winter temperature (WT), disturbance index (D), total nitrogen inputs (NTOT), the grass use index (GU) and the summer rainfall (SR) is presented in Figure.3.9a.

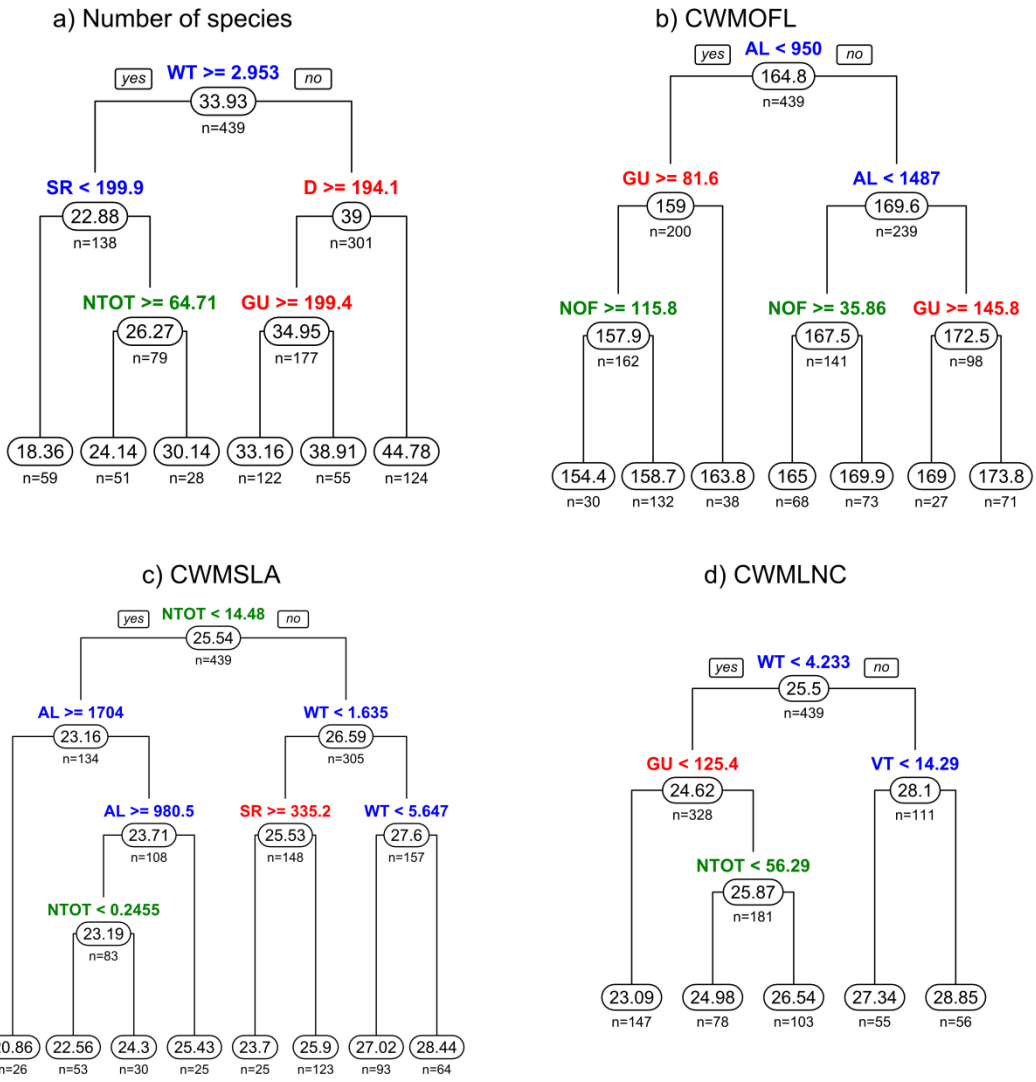


Figure.3.9: Regression trees on biodiversity variables from management and climate variables a) regression tree for Number of species computed from SR,WT,D,GU and NTOT; b) regression tree for CWMOFL computed from AL,GU and NOF .c) regression tree for CWM SLA calculated from NTOT, AL ,SR,WT , d) regression tree for CWMLNC calculated from NTOT, GU,VT,WT. In red the grass use variables, in green the variables related to the fertilization, in orange the variables related to the timing of use in orange. The value inside the circle is the mean value of each categories and the n correspond to the number of plots.

The first separation was due to the winter temperature (WT) with a threshold at 2.95°C. For the grasslands allocated in regions with a winter temperature above this threshold and with less than 200 mm of summer rainfall the average number of species was of 18.36 species. For the grasslands located in the regions also with a winter temperature above 2.95°C but with more than 200 mm of summer rainfall, another separation was made regarding the nitrogen inputs. Grasslands receiving more than 64.71 kg N ha^{-1} had an average of 24.14 species. Grasslands receiving less than 64.71 kg N ha^{-1} had 30.14 species. For the grasslands with a low winter temperature, two separations were made regarding the intensity of use (disturbance index or the grass use index).

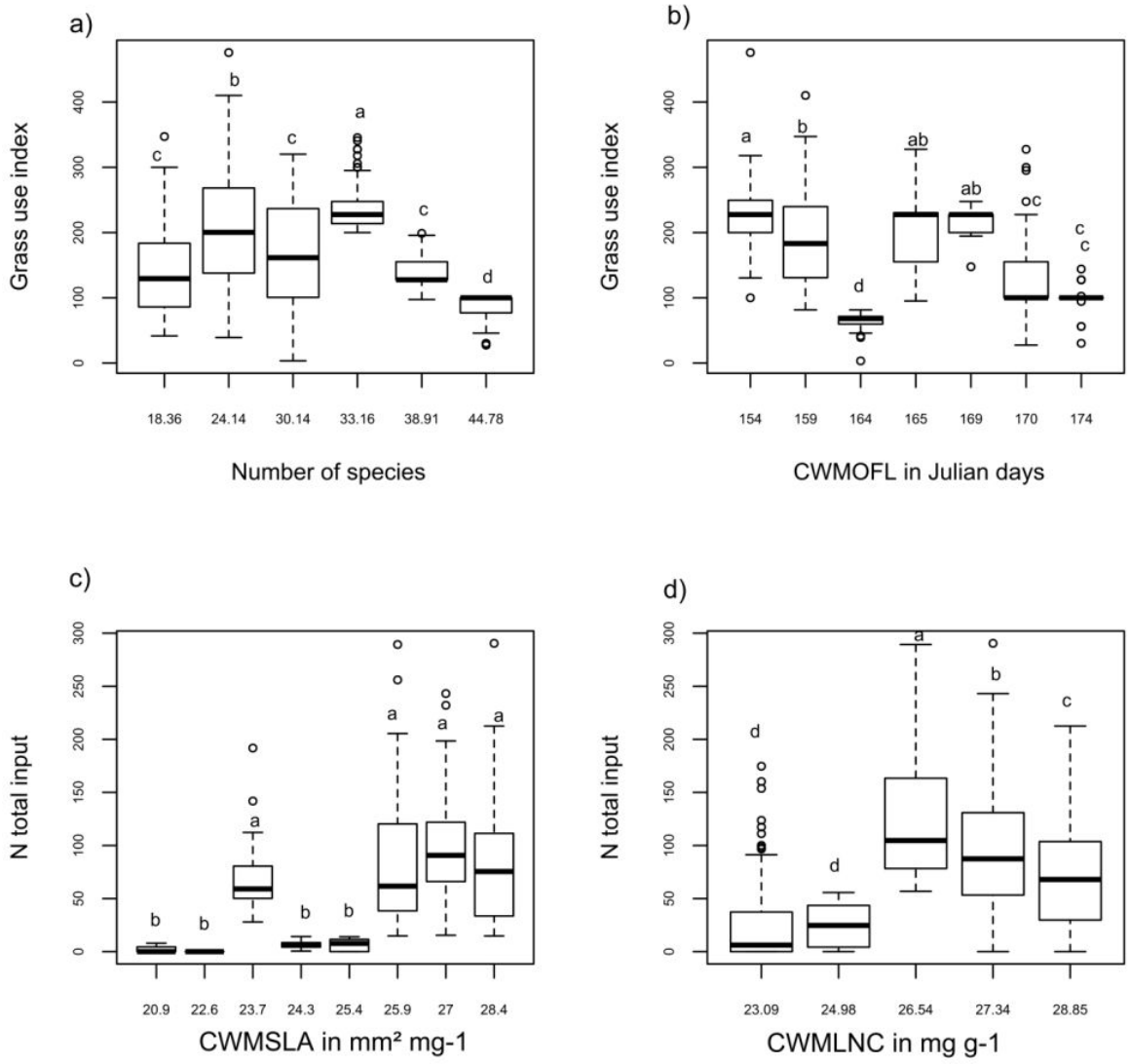


Figure.3.10: Difference in term of management between the different leaves of the regression trees. a) Effect of the leaves of the regression tree of number of species on the grass use index. b) Effect of the leaves of the regression tree of community mean value of onset of flowering on the grass use index. c) Effect of the leaves of the regression tree of community mean value of onset of flowering on the N total input quantity. d) Effect of the leaves of the regression tree of community mean value of leaf nitrogen content on the N total input quantity. The letters correspond to the difference between categories between multiple comparison tests after ANOVA.

For all the categories of the regression tree the difference in term of specific richness was significant (ANOVA) at the exception of the categories with an average of 30.14 and 33.16 species. For the difference in term of climate between the categories, the category 18.36 had a significant different climate of the other categories: lower altitude; lower rainfall and higher temperature. The categories 24.35 species and 30.67 species had medium climatic condition regarding the others categories. The three last categories had higher altitude and rainfall and lower temperature with sometimes difference between the three categories.
For the difference in term of management between the categories, the more intensive categories in term of management (GU and NTOT) were the 24.35 species and 33.16 species. The categories 18.36, 30.14 and 38.90 species had medium management intensity. The category 44.78 species had lower management intensity than the other categories (Figure.3.10a).

Regarding the study of the residuals effects within each category with the linear regression; we only found few effects mainly with climate and with a low percentage of explanation.

In the first category (18.36 species), no effect of management was found. In this categories the standard deviation of the different management variables was high (SD_{GU}=71.56; SD_{NTOT}=52.05).

To sum up, the number of species was not affected by the management in the lowland and the number of species decreases with an increase of the management intensity in mountain area

b. Onset of flowering

The regression tree of the community weighted mean value of onset of flowering was presented on the Figure.3.9b. It was made with altitude (AL), grass use (GU), nitrogen organic inputs (NOF) and sum of temperature at the first use (SDFU). First separations were due to the altitude with a separation at 950m and 1487m. In each of these three groups, the next separations were due to a management variable either by grass use index (higher altitude group) or by the nitrogen organic fertilization (medium elevation group) or a combination of these two variables (low elevation group).

In term of climatic difference, the categories 154.4, 158.7 and 163.8 Julian days for the flowering date had a warmer climate with lower altitude and less rainfall. Between these three categories, the category 158.7 Julian days was little more warm and lower altitude. The four other categories had a colder climate in altitude with more rainfall. The categories 169 and 173.8 Julian Days had a higher altitude. For the management difference, the categories 154.4, 158.7, 165 and 169.9 Julian days had a more intensive management in term of grass use intensity (Figure.3.10b) and organic nitrogen inputs than other categories. On the category 173.8, the nitrogen input (total and organic) had a negative effect on the onset of flowering (R^2= 0.14 for NTOT; R^2= 0.15 for NOF). It was the only effect found by linear model for the onset of flowering.

To sum up, the community weighted mean value of onset of flowering decrease with the management intensity in the lowland and in mountain area.

c. Specific leaf area

The regression tree of the community weighted mean value of specific leaf area is presented on the Figure.3.9c. It was constructed with the altitude (AL), total nitrogen inputs (NTOT), winter temperature (WT) and summer rainfall (SR). At the end of the regression tree, 8 different categories of specific leaf area (SLA) were presented.

The first separation was based on the nitrogen total inputs (threshold at 14.48 kg N ha^{-1}). Almost all the other separations were due to climatic variables expect for the low nitrogen inputs grassland between 980.5 m and 1704 m where a separation was made between the unfertilized and the low fertilized. For the differences between the categories in term of climate, the category 28.44 had a warmer winter, lower altitude and lower rainfall. The categories 25.43 and 27.02 have similar climate. The category 25.9 presented similar climatic conditions according to the two previous cited categories. The other categories had the close the same climate. For the management intensity, the main differences were on the nitrogen input between the categories 28.44; 27.02, 25.90 and 23.7 and the other categories (Figure.3.10c). In the category 25.43, the SLA was influenced by the climate (negatively with the altitude and the rainfall and positively with temperature). In the category 22.56, SLA was influenced by the rainfall. Nitrogen inputs have positive effect on the SLA in the categories 23.7, 25.9, 27.02 and 28.44.

d. Leaf nitrogen content

The regression tree for leaf nitrogen content was presented in the Figure.3.9d. It was run using the winter and vegetative temperature, grass use index and nitrogen total inputs. The first separation was with the winter temperature (4.23°C). For the warmer grasslands, the next separation was also with the temperature but with the vegetative temperature (14.29°C) Form the colder grassland during the winter, two separations were made regarding the management: first by the grass use index (125.4) and then by the nitrogen total inputs (56.29 kg N ha^{-1}).
The categories 27.34 and 28.85 had a warmer climate with less rainfall in lowland in comparison with the categories 23.09; 24.98 and 26.54.
For the grass use index, the categories 24.98, 26.54 and 27.34 were more intensive than the categories 28.85 and 23.09. The categories 23.09 and 24.98 had the lowest nitrogen input: then, the category 28.85, the category 27.34 and the category 26.54 (Figure.3.10d). No significant relationships inside the categories were found except for the category 26.54 with a negative effect of altitude.

The results of the different analysis performed on the dataset resampled from the entire dataset for better distribution of the altitude were similar to the results obtained with the dataset without resampling (results not shown).

D. Discussion

1. Conditional effect of management and climate on diversity

The responses of the number of species to the management intensity were **different between the grasslands from regions with a cold climate and the ones from regions with a warm climate**. For the colder grasslands (mountain grasslands), the intensity of defoliation had a negative effect on the number of plant species (Figure.3.9a). The negative effect of defoliation intensity and nitrogen inputs on the number of species is well known (Gaujour *et al.*, 2012).
For the warmer grasslands, the water limitation during the summer seems to be an important factor. However, the separation with the summer rainfall correspond also a separation with the other climatic variables regarding the large covariance between the climatic variables.
For the grasslands with the lower altitude, lower summer rainfall and higher temperature, **the number of species was not affected by the management**. The management of these grassland was very diverse in term of grass utilization (from 41 to 347) or in term of nitrogen inputs (from 0 to 199 kg N ha^{-1}), but the average management was not the most intensive of the dataset (Figure.3.10a). Within these groups, no effect of management was found even if the management was very variable within a group. The absence of effect of management for the grasslands with the most favorable climatic conditions was also found for other criteria (community weighted mean value of leaf nitrogen content). We discuss this absence of effects in a paragraph below.
The management also affected the onset of flowering. **Onset of flowering is earlier in disturbed and fertilized grasslands** (Figure.3.9b, Figure.3.10b). An early flowering is a viable strategy under frequently disturbed conditions (ruderal strategy). An early flowering allows the species finishing their reproductive cycles before the defoliation (Grime, 1974; Grime, 2006). The competitor and stress tolerant species have a later flowering due to the longer duration to the maturity (large and tall individuals for the competitor and lower growth for the stress tolerant). The onset of flowering was also related to the plant economics spectrum (Navas *et al.*, 2010). Acquisitive species have a quicker onset of

flowering and nitrogen inputs favor acquisitive species (Ordoñez *et al.*, 2009) and so species with earlier onset of flowering.

The onset of flowering was strongly affected by the climate, particularly by the altitude (Figure.3.9b). The onset of flowering is known to be related to the altitude especially within a species (Fitter and Fitter, 2002). Here, we only assessed the effects on the onset of flowering due to changes in species presence and abundance between communities. We can thus assume that the effect of climate on the onset of flowering would have been even greater if the intraspecific variability of this trait would have been considered in the computation of the community mean value.

The onset of flowering was measured in Julian days, which limits the interpretation of the results along a large climatic gradient. Indeed, a species always measured under mountain conditions would have a later onset of flowering than a species recorded in lowland areas even if these species have the same physiology and reproductive cycles. The sum of temperature in degree-days for the onset of flowering could be a better unit of measurement (Michaud *et al.*, 2012b). However, this functional trait is not available for the moment in the functional traits databases (Kattge *et al.*, 2011). The effect of climate may be overestimated due to the use of Julian days instead of another metric.

The **specific leaf area was influenced by the climatic condition and by nitrogen inputs** (Figure.3.9c). The main impact of management was a difference between grassland with almost no nitrogen inputs and grassland with nitrogen inputs. The rest of the regression tree was explained only by climatic variables (Figure.3.9c). However, the specific leaf area increased with nitrogen inputs in good climatic condition in our results. The specific leaf area results from a combination of climate factors and nitrogen inputs. In our regression trees, SLA is mainly defined by stress following the definition of Grime (Grime, 1974; Grime, 2006): a factor that limit the growth of the vegetation.

The negative effect of summer rainfall is contradictory with the know results on the positive effect on water availability on SLA (Wright *et al.*, 2005b; Ackerly and Cornwell, 2007). This effect may be due founding effect of other climatic factors (temperature or altitude) because of the high covariance between the climatic variables (Figure.3.6). Another hypothesis is that water limitation during the summer selects species with a quick vegetative cycle enabling them to avoid the dry period. The species with quick vegetative cycle have higher SLA (Navas *et al.*, 2010).

In the regression tree for leaf nitrogen content, highest values of the **leaf nitrogen content** were **only defined by the climatic conditions** (Figure.3.9d). The leaf nitrogen content is related to the photosynthesis activity (Wright *et al.*, 2004). The limitation of the photosynthesis by the water and the temperature are well known. The effect of climate on LNC can be due to the limitation of photosynthesis. Leaf nitrogen content was also defined by management only for the grassland with a colder winter. In colder conditions, the leaves nitrogen economics spectrum was defined by the defoliation. Defoliation favors species with quick growth and so a higher LNC. Under low disturbance, stress tolerant and competitor are more presents. They have lower growth and so lower LNC.

Our results show also that some conditional effects occur (i.e. effects of one variable in function of another variable).Indeed, the management affected the functional diversity only in some climatic conditions like in de Bello *et al.* (2006). In the next paragraph, we proposed hypotheses regarding the absence of management effect under some climatic conditions.

2. Absence of management effect in some climatic conditions

The category with the **lowest number of species and the one with the highest leaf nitrogen content community weighted mean values were only defined in the regression trees by climatic variables**. Furthermore, no management effect was found inside these categories of the regression trees even if the management was very variable.

The absence of management effect concerned 59 grasslands for the number of plant species and 111 grasslands for the leaf nitrogen content including the 59 grasslands of the number of plant species. In these 59 grasslands, 106 species were present. The percentage of grass species was higher in these 59 grasslands (81%) than for the 439 grasslands in the dataset (60%). These grasslands were mainly localized in the north part of France and all originated from the French national typology dataset (Michaud *et al.*, 2012b).

The absence of management effect on the number of species and leaf nitrogen content was for the good climatic conditions in term of temperature. This result was quite surprising. The absence of management effects in very hard climatic conditions (dry or very cold condition) would have been more logical. Under adverse climatic conditions the variability of management would be very restricted by the climate. Furthermore, for some authors, the effect of management on biodiversity is higher on productive grassland (Milchunas and Lauenroth, 1993; Janeček *et al.*, 2013). However, we can make some hypotheses to explain the absence of management effects for these good climatic conditions.

One hypothesis for the difference between warm and cold grasslands for number of species and leaf nitrogen content could be that the management is more intense in good climatic conditions. However, it was not the case in our dataset (see Figure.3.9ad). This hypothesis cannot explain the difference observed between high and low grasslands.

The **history of management** may be another explanation to the absence of effect of management. We may state that in the lower lands the past management was more intensive than in higher lands for two different reasons. First, in altitude, grasslands are the only available type for agriculture. In lowland, all the types of agriculture are possible. Furthermore in France, the percentage of grassland was very low in the middle of XIX century (4198000 of ha in 1842 versus 13324000ha in 1950) (Huyghe, 2009). We may suppose that the probability that grassland was a crop is high in the lowland.

Secondly, good climate in these regions favors a grass production and so allows intensive defoliation. However, some recent regulations induce a limitation of the management for example due to the imposition of ecological compensation area.

After a period of intensive management, soil seed pools are generally poor in species diversity. The soil seed pool is an important source of species (Gaujour *et al.*, 2012). Without soil seed pool or other dispersal mechanism, the colonization of new species would be very difficult especially if no other sources of species are not available. **The history of management could be a plausible hypothesis for the number of plant species.** However, for the community weighted mean value of functional traits, several studies have shown a variation with a change of management (Garnier *et al.*, 2004; Louault *et al.*, 2005; Laliberté *et al.*, 2012) even without the apparition or disappearance of species. For example, the community weighted mean value of specific leaf area decrease during an extensification (Louault *et al.*, 2005). Soil seed pools have also an effect on the functional characteristic of the vegetation (Pakeman and Eastwood, 2013).

Landscape could also **explain the lower specific richness** found in the regions with the warmer temperature. Using the CORINE land cover obtain from MODIS (500m of resolution), the grasslands in lowland were mostly crops dominated landscape or within a mixed crops natural vegetation mosaic landscape. At the opposite, the highland grasslands were mostly in forest dominated landscape and some in grassland dominated landscape. The composition and the structure of the landscape is one driver of the specific richness in grasslands (Gaujour *et al.*, 2012). The presence in the landscape of species sources could be important especially during an extensification for the colonization of new species. The effects of landscape on functional trait/identity/diversity are poorly known for the moment.

The last hypothesis is that the climate has a strong influence **on soil processes**. In warm grasslands, the nutriment recycling is important and so soil fertility would be high even without fertilization. Leaves economics spectrum traits like LNC and number of species are very sensitive to the N soil availability. Moreover, high LNC vegetation is related to a quick decomposition of the litter (Freschet *et al.*, 2012) and so quicker soil process (Grigulis *et al.*, 2013). For the extensive grassland in these regions (1 cut or extensive pasture), the forage export and so the nitrogen export is low so the nutriments recycling could be enough to maintain an acquisitive species (high LNC) without any nitrogen inputs. The Ellenberg fertility index was higher in the categories of regression tree with high LNC.
The three last hypotheses we made to explain this absence of effect of management in warmer grassland seems also relevant for the specific richness. However for the community weighted mean value of leaf nitrogen content, the actual knowledge is still to limited to be able to conclude on the two first hypotheses.

E. Conclusion

For some criteria like the number of species or the functional identity of leaf economic spectrum traits and reproductive traits, **we can suppose that the random forest calculated from the management and the climate could be used to estimate functional diversity criteria.** For some criteria, however, the estimation was very bad like for the functional dispersion criteria. The influence of management and climate can be poor on these criteria. Climate variables had more importance on the estimation of functional identity and number of species than management. Climate is the first ecological filters and management only affects functional trait value and species after the climate (Keddy, 1992). However, climate variables are more accurate than the management variables. Indeed management variables relied on farmers interviews and some approximations were made to obtain the aggregated variables like grass use index. The effects of climate and management are not continuous. Indeed, some conditional effects occur in the links between vegetation, climate and management. Regression tree family analyses are able to identify this kind of effects. The percentage of variances explained by the stepwise was lower of around 5% for the four criteria in comparison to the random forest. The main conditional effect found was that under good climatic conditions.
Conditional effects on the vegetation need further studies. **Adding historic management, landscape and soil variables** would be a step forward on the estimation of functional diversity and number of plant species

VI. Chapter conclusion

A. Conclusions on the effects of management and climate on functional diversity

Most of the hypotheses tested (cf Introduction) were confirmed by our results.

- The onset of flowering was later under adverse climatic conditions (high altitude low temperature) and in extensively managed grassland (low nitrogen inputs and infrequent defoliation).
- The specific leaf area and leaf nitrogen content were higher in good climatic conditions and intensively managed grassland, as against the number of plant species.

The originality of our work was more on the evaluation of the relative strength of each factor. We found that climate had a stronger effect than management for the number of plant species and the onset of flowering. For the leaf nitrogen content and the specific leaf area, the management and the climate (alone) had a similar strength. Some functional

diversity criteria are more influenced by the management than others. This relative strength depends also on the gradients present in the dataset. We had a very large gradient of climate. The gradient in term of management may have been less pronounced. For example, we did not have plot with more than 3 cuts per year. We also found differences between the different climate variables and the different management variables. For some criteria, the effect of altitude was mainly due to the winter temperature like the leaf nitrogen content. On the contrary, the effect of altitude was not due to the effect of winter temperature for the onset of flowering. This kind of results should still to be confirmed by other studies and eventually by experiments.

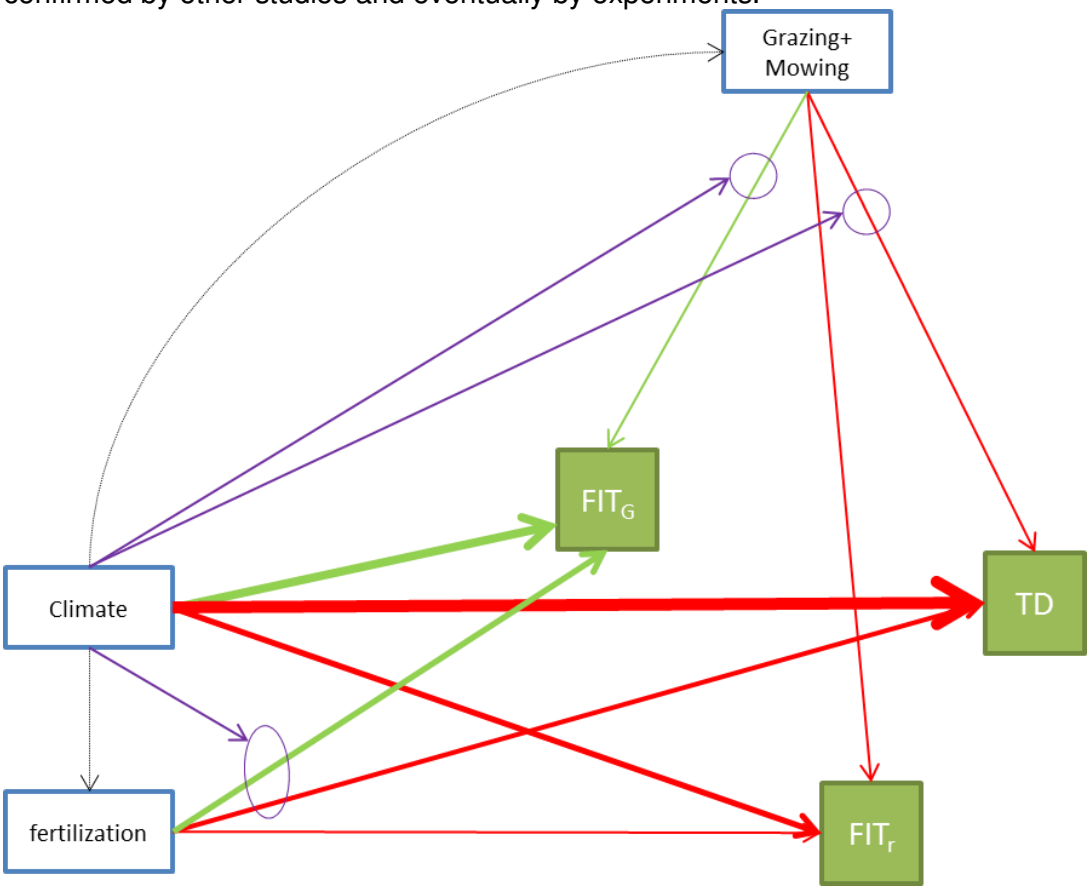


Figure3.11: Conceptual schema based on results on the effect of climate and management on functional diversity. FIT_G: Functional diversity of trait linked to the growth; FIT_R: Functional diversity of traits linked to the reproduction; TD: Taxonomical diversity. In purple, the effect of climate on the effects of management on the functional diversity (i.e. conditional effects); the red arrows , the negative effects of climate and management on functional diversity; the green arrows the positive effects of climate and management. The strength of the arrow is proportional to the strength of the effect identified in the Structural equation models.

We also found conditional effects like in de Bello *et al.,* (2005). The effect of management differs between climatic conditions. We find conditional effects only for the number of plant species and the leaf nitrogen content as well the specific leaf area. We update the conceptual model propose in the introduction (Figure 6) in the figure 3.11.

The presence of conditional effects shows the importance of interactions between factors. This is logical regarding the ecological filters theory proposed by Keddy (1992) on the effect of the environment on functional traits. The climate first selects some traits values. The soil, the management and the competition then select some other values.

For the specific richness, we suppose that conditional effects occur especially when the biodiversity is degraded. For example, in the case of extensification, sources of species are necessary for the comeback of new species like landscape and seed sources.

The most original result we found was the absence of management effects on the number of plant species and leaf nitrogen content in the grasslands with a warm climate. Even if this result is surprising, we can find some explanations to these results. These explanations are based on unmeasured factors in our study like soil fertility, landscape and soil seed bank. We propose an amelioration of the conceptual model adding some new hypotheses (Figure 3.12).

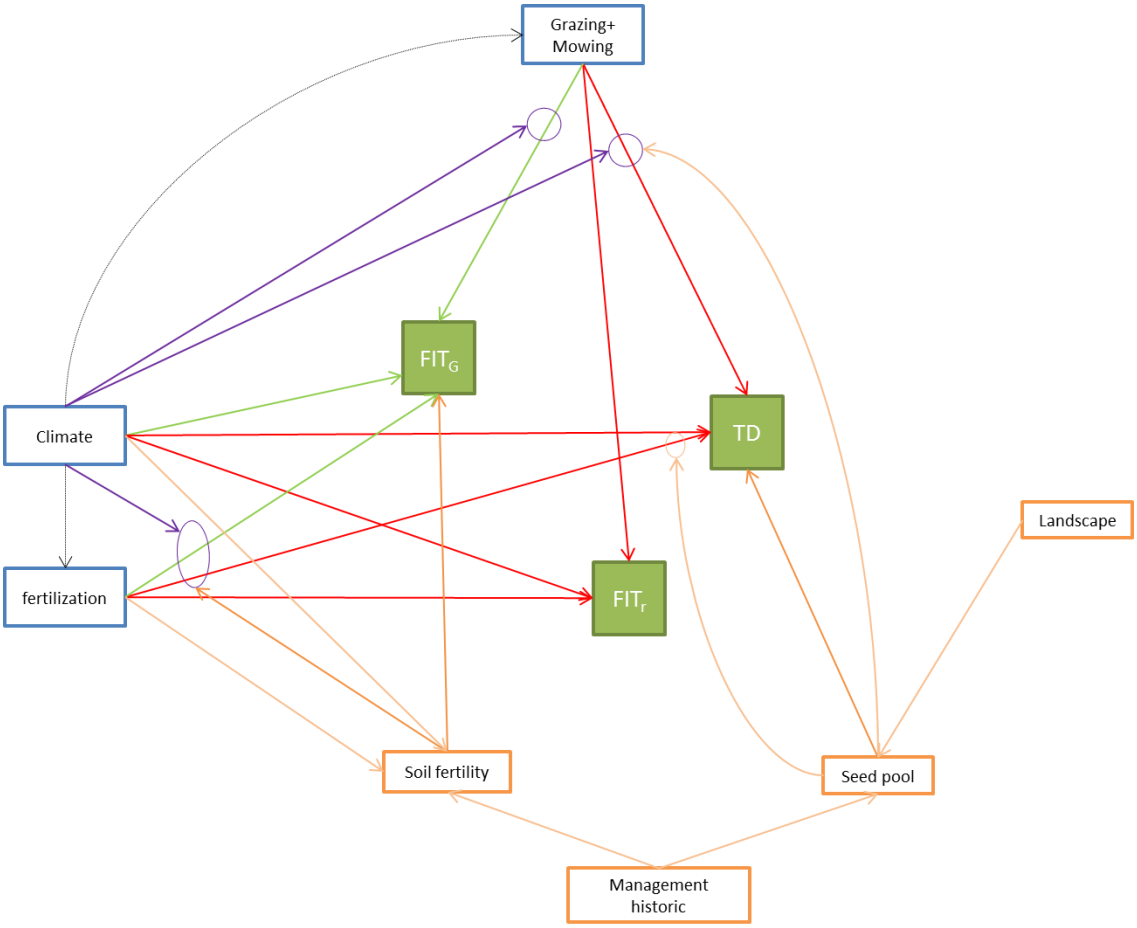


Figure3. 12: Conceptual schema based with new hypothesis after the results of the effect of climate and management on functional diversity. FITG: Functional diversity of trait linked to the growth; FITR: Functional diversity of traits linked to the reproduction; TD: Taxonomical diversity. In purple, the effect of climate on the effects of management on the functional diversity (i.e. conditional effects); the red arrows , the negative effects of climate and management on functional diversity; the green arrows the positive effects of climate and management. In orange, the new hypotheses regarding the explanatory factors on biodiversity

B. General trends versus conditional effects

We tested two types of effects in order to estimate functional diversity criteria. We can so compare the results of these two types of analyses. First, the structural equation models tested were not well fitted according to the different indicators used (Hooper *et al.*, 2008). So, the structural equation models (SEM) tested cannot be used to estimate grassland plant diversity. For the random forest, no indicators other than the percentage of variances explained exist to conclude on the quality of the analysis.

If we compare **the R^2 of the structural equation model and the percentage of variances explained** by the random forest, we observe that the random forest had a stronger power of explanation. For example, the R^2 of the SEM for the number of species was of 0.48 (i.e. 48% of variance explained) versus 61.57% of variance explained for the random forest. For the other criteria, the percentage of variance of the random forest and

the structural equation model were respectively for the SLA of 48.30% versus 43%, for the LNC of 42.5% versus 32% and for the onset of flowering of 42.07% versus 34%.

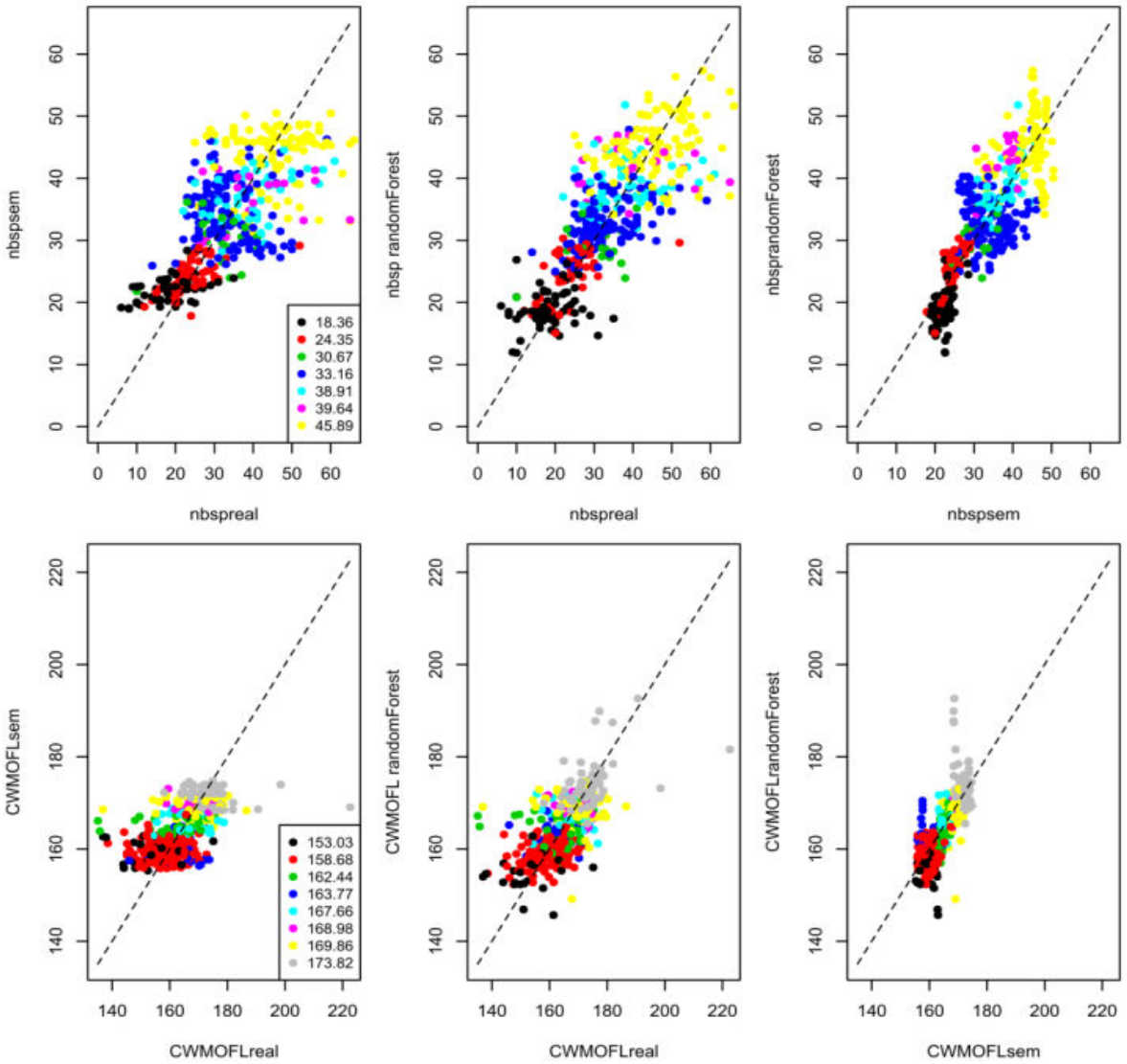


Figure.3.13: Relationships between the biodiversity criteria from the dataset , the predicted from the SEM and the predicted from the random forest. The first column of graphics presents in X the real criteria and Y the SEM prediction, the second column of graphics in X real criteria and Y the random forest prediction and the third column of graphics in X the SEM prediction and in Y the random forest prediction. The first line is the results for the number of plant species, and the second one for the functional identity of onset of flowering. The color represents the different leaves of the regression trees.

The Figures 3.13 and 3.14 present the relationships between the functional diversity criteria from the dataset, the predicted criteria from the SEM and the predicted criteria from the random forest. The structural equation model was less accurate for the extreme value in comparison with the random forest. It is very interesting to notice that the values predicted from the structural equation model and the random forests are strongly correlated. It means that **the non-explained variance in the two analyses seems to have the same origin**. The multiple regression trees in the random forest may so take account the general trends. The strong correlation between the two estimations supports the hypothesis that some important variables are missing (soil, landscape and history).The higher power of explanation of the random forest could be explain by the capacity to evaluate condition effects as for example the absence of management effect on the

vegetation in low altitude. However, the higher explanation may be due to the highest number of variables used for the random forest (20 versus 5 for the best structural equation model).

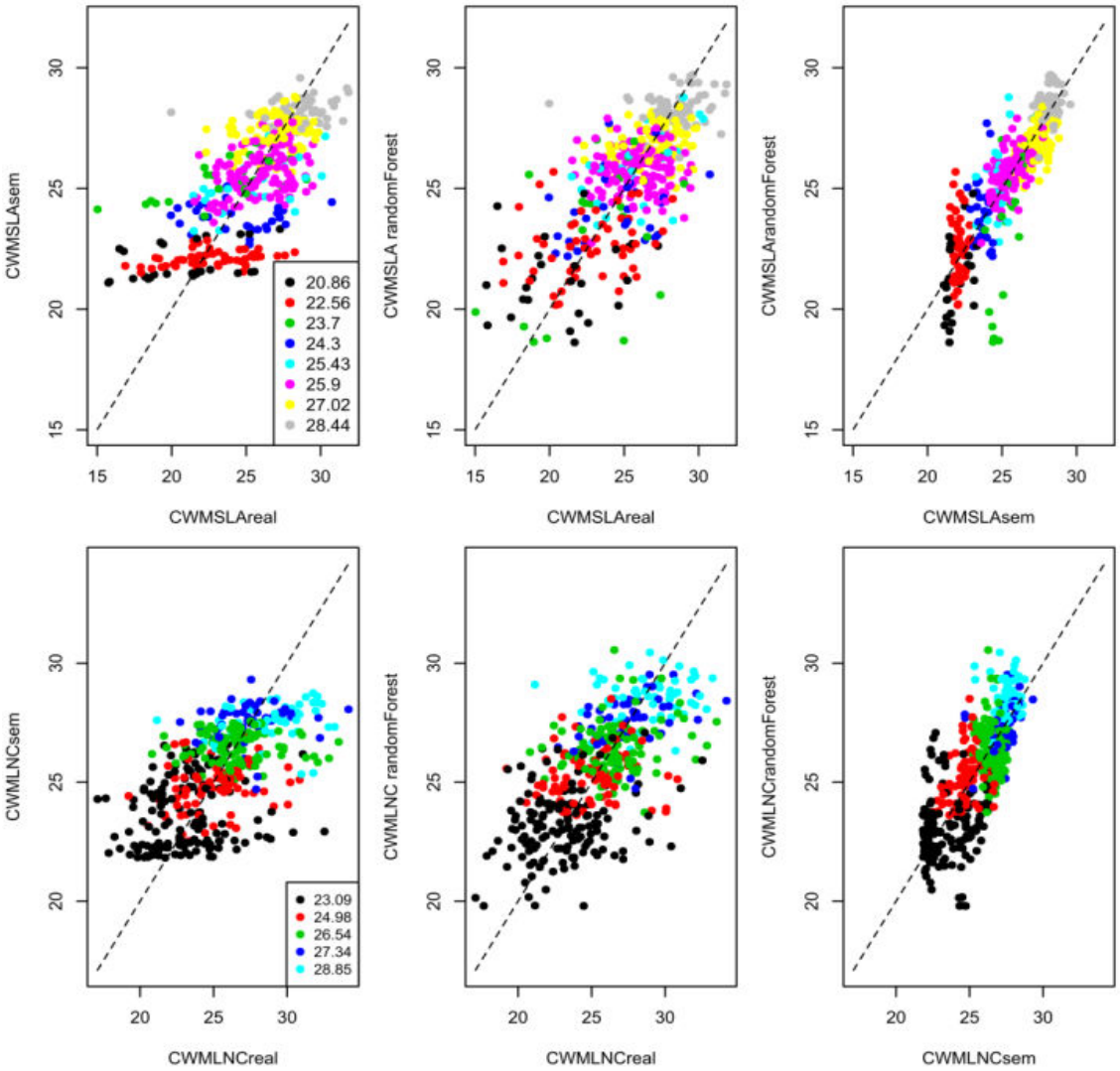


Figure.3.14: Relationships between the biodiversity criteria on the dataset (real one), the predicted from the SEM and the predicted from the random forest. The first column of graphics presents in X the real criteria and Y the SEM prediction, the second column of graphics in X real criteria and Y the random forest prediction and the third column of graphics in X the SEM prediction and in Y the random forest prediction. The first line is the results for the functional identity of specific leaf area and the last one for the functional identity of leaf nitrogen content. The color represents the different leaves of the regression trees.

The two hypotheses on the relationships between climate, management and vegetation seem to be partially true (general trends and condition effects). Globally, we observed some general trends. The effect of management and climate on functional diversity could be described by a **succession of conditional effect forming general trends**. The Figure.3.15 presents a 3D representation for the number of species regarding the intensity of management and climate defined by the structural equation model (representation of the general trends). The different groups issued from the regression trees were projected on the figure. The Figure.3.16 presents the same figure for the functional identity of SLA.
The similarity between the results of the different functional diversity criteria may be explained by relationships between them. The strong relationship between specific leaf

area and leaf nitrogen content are already shown in many studies. These two traits are good indicators of the leaf economics spectrum (Wright *et al.*, 2005a). The reproductive traits like the onset of flowering are also influenced by the leaf economics traits (Navas *et al.*, 2010). The link between the number of species and the functional identity criteria are not so clear. The drivers of the functional diversity and the number of species are the same. High SLA and LNC characterize also high competitive species. The competition may explain the low specific richness.

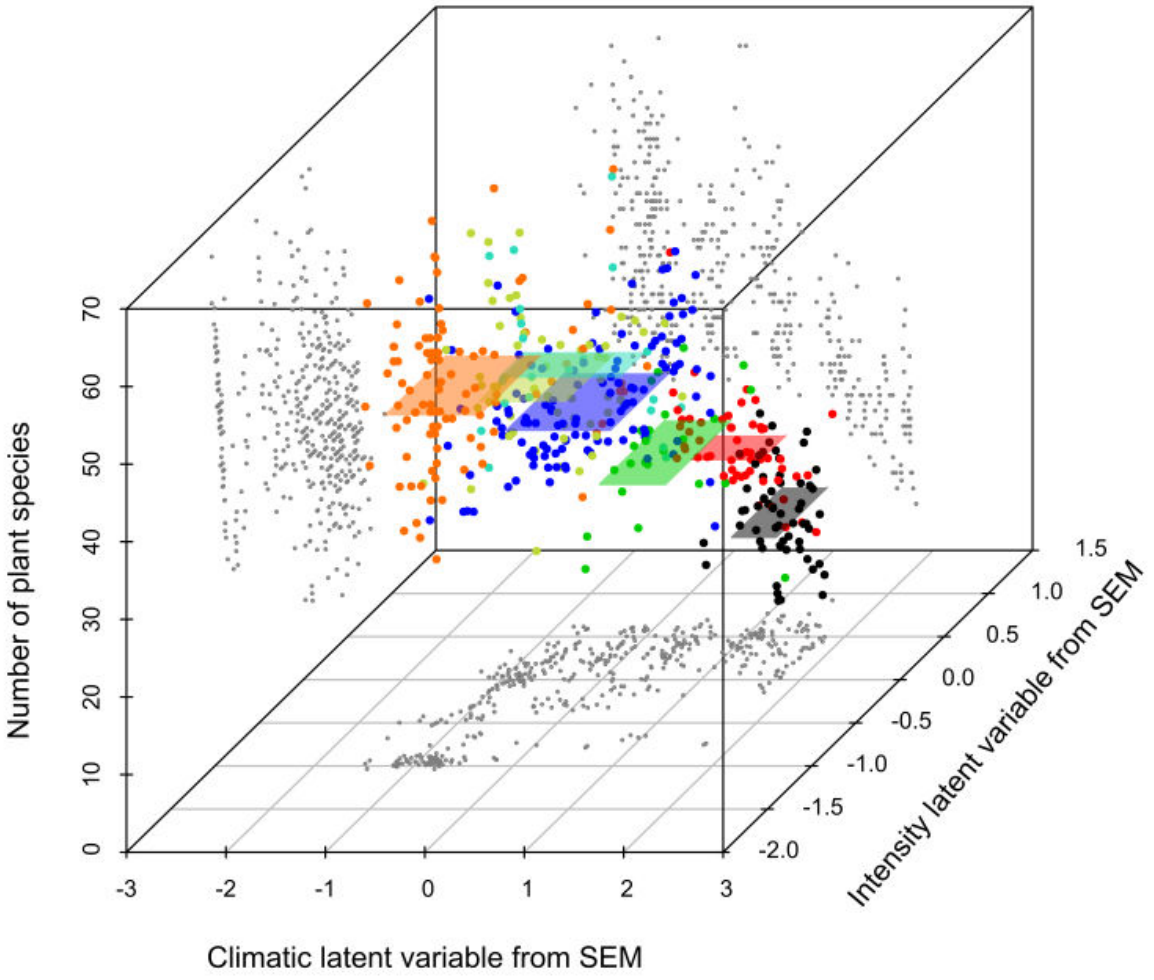


Figure.3.15: 3D representation of the intensity latent variable from the Structural equation model, climate latent variables from SEM and the number of species. The color of the points corresponds to their belonging to one of the leaves of the regression tree. The rectangle corresponds to the means value of the latent variables with +/- a standard deviation for each leaves of the regression tree.

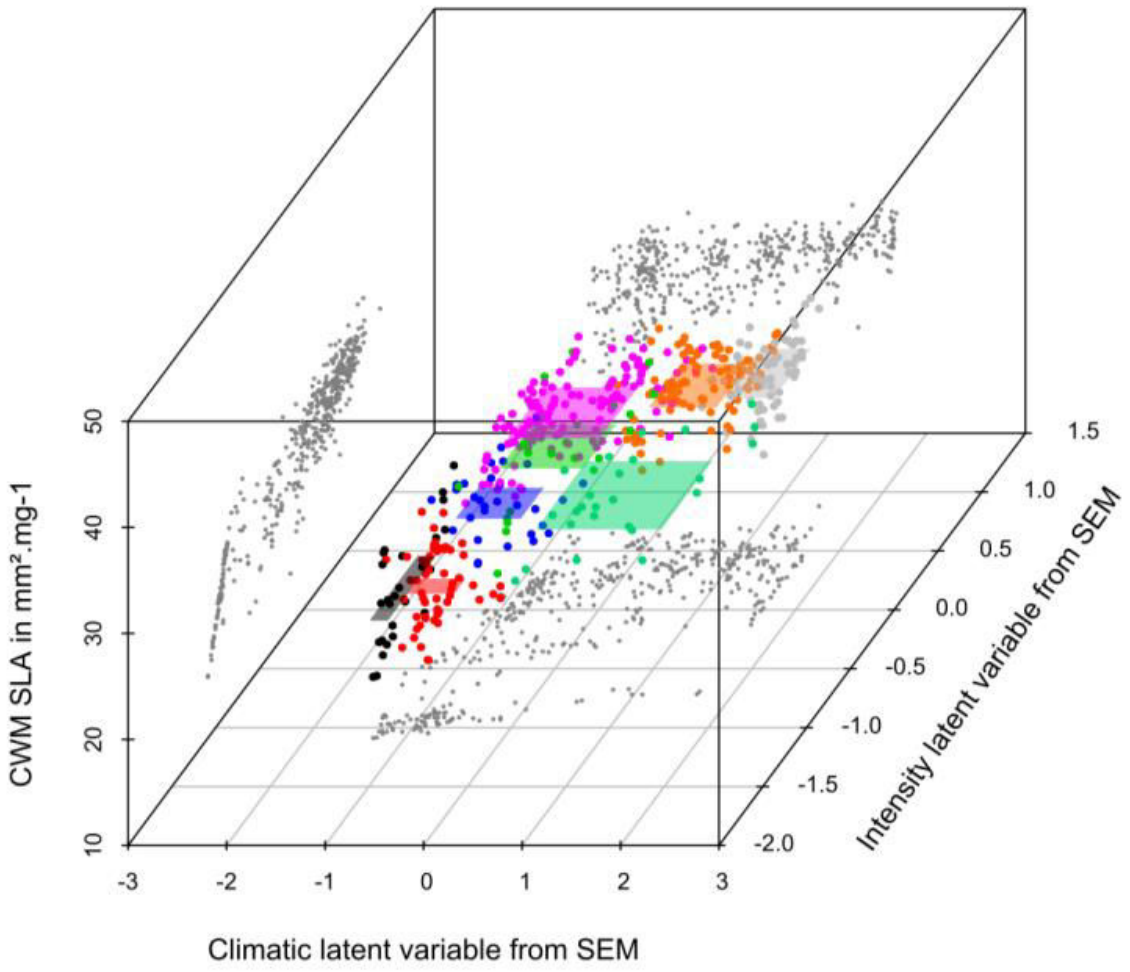


Figure.3.16: 3D representation of the intensity latent variable from the Structural equation model, climate latent variables from SEM and the functional identity of SLA. The color of the points corresponds to their belonging to one of the leaves of the regression tree. The rectangle corresponds to the means value of the latent variables with +/- a standard deviation for each leaves of the regression tree.

C. Results of the random forest on all the functional diversity criteria

The Table.3.13 presented the results of the random forest made on all the criteria selected in the first chapter. The six first most important variables of climate or management were also presented in the Table.3.13 for each criterion. Only 10 criteria of the 29 selected had a percentage of explained variance superior to 40%. The community weighed mean values had generally high percentages of explanation except for the LDMC (10.94%) and the vegetative height (20.07%). For the criteria based on the percentage of a functional group, the explanation was higher when the average and the standard deviation of the percentage functional groups were high on the dataset like the percentage of grass and forbs for example. Functional groups with low average relative abundance like percentage of legumes or percentage of apiaceous species were poorly explained. The poor explanation for the legumes (only 1.2%) was quite surprising. Indeed the legumes species response generally well to management and climate (Michaud *et al.*, 2012b). The criteria of diversity richness (number of species and functional richness) were generally well explained. Functional dispersion was poorly explained. The most important variable for all the criteria was a climatic variables generally the altitude. For some criteria like the percentage of forbs, the six first most important variables were only climatic variables.

D. Predicting functional diversity: why so much error?

For some variables, the percentage of explanation was very low. The others had generally only medium percentage of explanation. For all the criteria, a large part of the variance was not explained. This might be due to different reasons:
First, each variable (management, climate, biodiversity) used had some uncertainty. The management data originated mainly from farmers interview that might be incomplete. Sometimes, the declaration of the famers was poor. Some variables of management were not directly recorded like the grass use index but indirectly estimated by the stocking rate and the number of cuts per year (Annex II). These transformations induce some errors. For the nitrogen inputs and the grass use index, we used general coefficients to transform the raw data from the farmer's interview. Such general coefficients do not take the specificity of the farm into account and are therefore bound to some errors.
The climatic variables were certainly more accurate especially the altitude. It may be a reason of the high explanation power of the climatic variables in the random forests. The variables of functional diversity were also computed with some bias due to the use of functional trait database and the difference of protocol between the different datasets (see Chapter 2). The very low percentage of explained variance for the functional dispersion indexes can be related to the low robustness of these indexes to intraspecific variability and sampling area (Chapter 2). The CWM is more robust to the different bias tested in chapter 2 and also better explained by climate and management. The intraspecific variability of the vegetative height varies is high that could explain the low percentage of explanation of CWMH. LDMC does not vary a lot inside a species. The lowest percentage of explained variance of CWMLDMC results of other unknown process.
The low percentage of explanation observed for some criteria could challenge the evaluation of ecosystem services from management and climate

Table.3.13: Results of the random Forest (see table 1.4 for the abbreviation)

Functional Criteria	**%variance**	**Importance**
%forbs	35.23	SR/AL/YR/WT/VT/PR/
% Grass	31.95	AL/SR/WT/NTOT/VT/PR
% legumes	1.2	YR/SR/WT/SDFU/VT/AL/
Legumes grass	0	AL/NTOT/VT/NOF/WT/D
% apiaceous	8.28	WT/SR/AL/YR/VT/SDFU/
%dicot	32.11	AL/SR/VT/WT/PR/NTOT
%Bee sp	1.69	SR/WT/PR/AL/VT/DFU
%Bumblebeessp	12.71	SR/PR/AL/YR/VT/WT
% Butterflysp	0	D/AL/YR/GU/VT/RGU
% Bumblebeessp legumes	0	NTOT/GU/SR/AL/WT/DFU
Beenbsp	67.01	SR/DATA/WT/PR/RGU/AL
Bumblebeesnbsp	67.09	SR/NTOT/DATA/YR/AL/PR
Butterflynbsp	30.3	NTOT/SR/AL/DFU/YR/WT
Number sp	61.57	AL/WT/NTOT/SR/NOF/YR
CWMH	20.07	AL/WT/NOF/NTOT/SDFU/PR
CWMLDMC	10.94	AL/RGU/SR/D/GU/WT/
CWMLNC	40.76	WT/AL/GU/SR/YR/D
CWMOFL	41.5	AL/VT/WT/DFU/NTOT/SR
CWMSLA	46.94	AL/NTOT/WT/SR/DFU/RGU
FDH	8.07	AL/PR/SDFU/WT/DFU/NTOT
FDSLA	7.89	WT/NTOT/DFU/AL/NOF/GU/
FEH	58.06	WT/PR/VT/GU/DFU/D
FRH	14.88	SR/AL/SRPY/VT/WT/RGU
FRLDMC	39.73	AL/D/PR/SR/WT/GU
Ellenberg Temperature	86.15	WT/AL/SR/VT/PR/RGU/
Humidity Ellenberg	51.05	AL/RGU/WT/SR/VT/NTOT
Flower duration	49.89	NU/D/RGU/DFU/AL/GU
Nectar quantity	0	PR/SR/WT/RGU/AL/NU
Pollen Quantity	15.98	SR/AL/VT/SDFU/YR/DFU

General Discussion

I. Principal conclusions of the different chapters

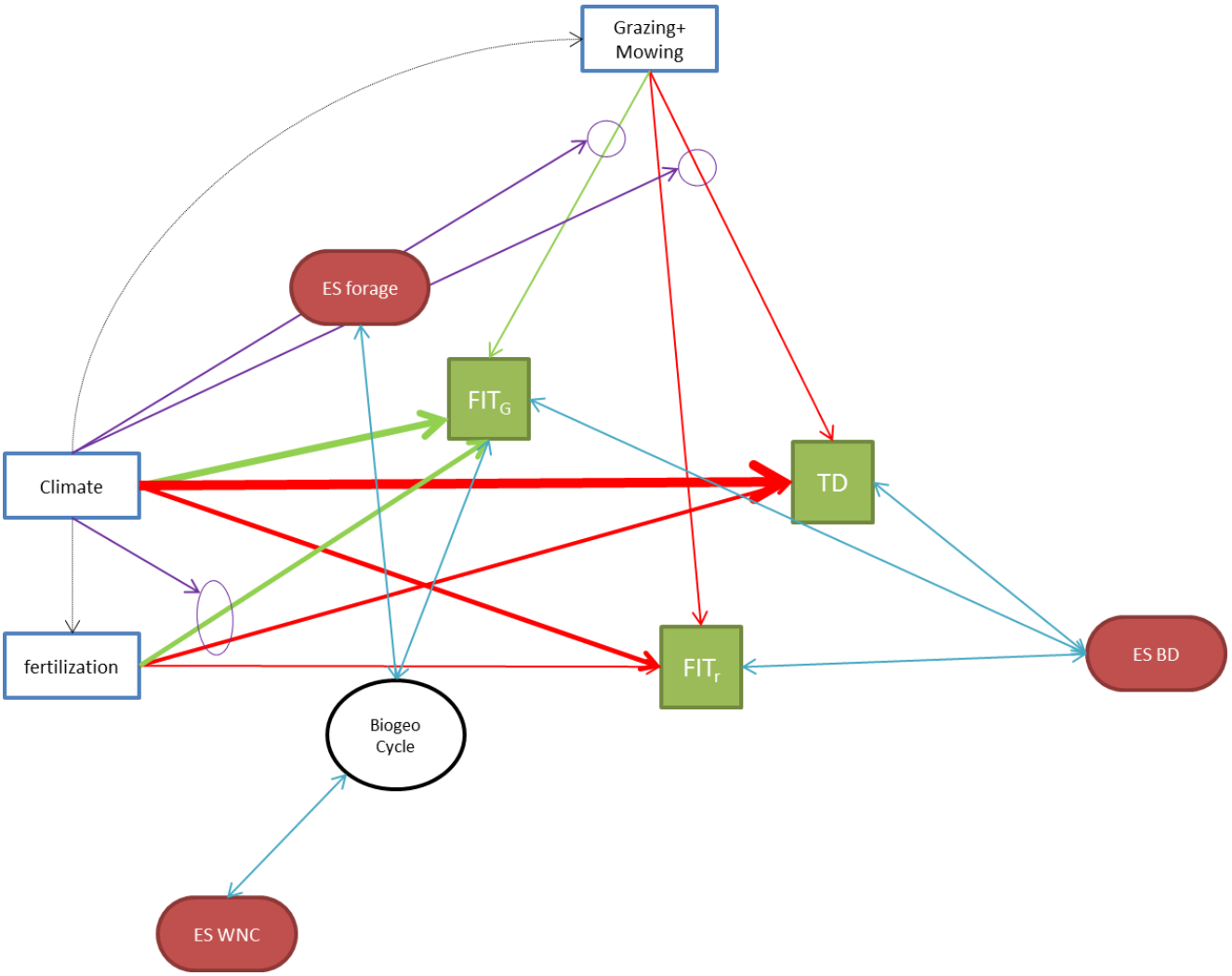
In this section, I have summarized the conclusions of each chapter of the PhD. From these conclusions, I have attempted to propose a conclusion for the overall objective of the PhD: **assessment of the effect of management and climate on the plant functional diversity related to ecosystem services of grasslands**.

The objective of the first chapter was to select functional diversity criteria that are useful when evaluating ecosystem services. This chapter relies on the hypothesis that functional diversity criteria with a strong links with ecosystem services can be found. I proposed a list of 29 different functional criteria related to 8 ecosystem services delivered by permanent grasslands. Some of the links between these 29 criteria and the ecosystem services were reviewed and agreed with various experts. However, other links between these criteria and ecosystem services were weaker. These weaker links were, in some cases, due to the absence of studies between some process and functional diversity. For the other links, the weakness was due to the limited availability of more related functional criteria, and therefore, the utilization of other functional diversity criteria as a proxy indicator.

In the second chapter, the objective was to assess the possibilities and limits of calculating functional diversity with functional trait values extracted from functional trait databases and by using various sources of surveys. One hypothesis tested in this chapter was that these errors were due to the existence of missing data or by the intraspecific variability. In the case of missing data, I tested different methods of imputations and proposed that a functional diversity threshold could be calculated with up to 30% of the data missing. For the intraspecific variability, I proposed a framework to evaluate the influence of intraspecific variability on functional diversity. The outcomes of these two studies will improve utilization of functional trait databases. In addition, I tested a second hypothesis in this chapter, predicting that we could regroup surveys with different protocols without inducing much error. Some of the 29 functional diversity criteria were only slightly influenced by these errors. The remaining criteria may be considered of poor quality of their calculations from functional trait databases.

The objective of the third chapter was to evaluate the effects of management and climate on twenty nine functional diversity criteria. The hypothesis tested in this chapter was that functional diversity of grassland is largely determined by climate and management within the range of grasslands available in the database. Two types of relationships between management, climate, and the functional diversity criteria were found: “general trends” (effects of a variable that are independent of the values of other variables) and “conditional effects” (effects of a variable that depend on the value(s) of one or more other variables). The evaluation using conditional effects yielded more conclusive results than that based on general trends. Furthermore, we found that in the warmer climates sampled in this study (low elevation); management did not significantly affect certain functional diversity criteria (such as the number of plant species and the functional identity of leaf nitrogen content). Climate had a stronger effect than management for most of the criteria. For the criteria based on the number of plant species or on functional identity of some traits, climate and management both had a strong influence (more than 40 % of the variances explained by management and climate information). Other criteria were poorly explained by management and climatic variables. However, even for criteria that were poorly explained by these variables, estimation of the functional diversity criteria from

management and climate conserves the general differences in functional diversity criteria between grasslands. Criteria, that require consideration regarding their calculation from functional trait databases (Chapter 2), were generally poorly explained by climate and management.
The proposed conceptual model in the introduction (see Figure 7) can be updated with results of the different chapters of this PhD (see Figure D1).



FigureD.1 Conceptual schema based on new hypothesis, following the results of the effects of climate and management on functional diversity. FIT$_G$: Functional diversity of trait linked to growth; FITr: Functional diversity of traits linked to reproduction; TD: Taxonomical diversity. In purple, the effect of climate on the effects of management on functional diversity (i.e. conditional effects); the red arrows , the negative effects of climate and management on functional diversity; the green arrows the positive effects of climate and management. In orange, the new hypotheses regarding the explanatory factors on biodiversity. ESFORAGE: Ecosystem services related to forage production; ESWNC: Ecosystem service related to the nitrogen, carbon and water cycle; ESBD: Ecosystem services related to biodiversity conservation.

The management and climate variables have a strong effect on some functional diversity criteria related to ecosystem services. Estimation of functional diversity from management and climate information could offer an important improvement in evaluation of ecosystem services of grasslands.

II. Evaluation of ecosystem services from management using functional diversity

At the origin of the project, the evaluation of ecosystem services from management using functional diversity was the initial objective of this PhD. The primary objective of the interviews made in the first chapter was to develop indicators of ecosystem services using functional diversity as inputs.
In parallel with my work, indicators were developed for forage and biodiversity services. These indicators were constructed by Dr Rosalinde Van Couwenberghe for the forage service and by Prof Sylvain Plantureux and Dr Bertrand Dumont for the biodiversity services. The indicators were developed using decision trees with a fuzzy partitioning model. In a decision tree, variables are successively aggregated following aggregation rules in the form of "IF and THEN" (for example, IF the variable A is >Xa, IF the variable B is<Xb, THEN the aggregation is equal to Y). With a classical decision tree, only categorical variables (or continuous variables with thresholds) can be aggregated. The choosing of an appropriate threshold is problematic. Fuzzy logic can be used to smooth the transition around the threshold, making more continuous aggregation. Fuzzy partitioning makes it possible to account for uncertainty in the decision boundaries between the various alternatives. The indicators were developed using FISPRO software. At the conclusion of this PhD project, only some prototypes of these indicators were available for the biodiversity services. These indicators will eventually be modified, based on validation using real data. Furthermore, the objective of the indicators changes slightly during their construction. For biodiversity services, the final objective was to have the best predictor of abundance and diversity of the different animal's taxa using functional diversity and management information, rather than functional diversity information alone. For forage indicators, the objective was more focused on assessing the ecosystem benefits (such as the possibility for winter stocks, or possibility of grazing during the summer), rather than ecosystem services (such as the quantity or composition of forage). Furthermore, as for biodiversity services, functional diversity variables were not the only inputs of the indicator. Other variables, such as soil characteristics, were also used as input.
For the services related to biogeochemical cycles, another method was applied. A biogeochemical model was created following the conceptual model of biogeochemical cycles proposed in the first chapter of this PhD. The model was based on pools of carbon, water, and nitrogen, and the flux between the pools. Each flux was defined from functional diversity criteria. The model created was very complicated; indeed more than 70 parameters had to be used to run the model. Furthermore, the values for these parameters in the literature were largely incomplete in literature, and in no data were available to fit the model. Therefore, we were unable to use the model to produce indicators for these services.
The objective was to combine these evaluation tools with the estimation of functional diversity from management and climate, presented in Chapter 3, from which we could examine the lack of information with respect to evaluation of ecosystem services when the functional diversity criteria are not measured but estimated from management and climate.
In situations where the loss of information was low, this method could be a way to effectively evaluate ecosystem services of permanent grasslands. However, this method does not account for the direct effect of management and climate on ecosystem services. In the framework proposed by Diaz *et al*. (2007a), the effects of management and climate on ecosystem service are evaluated first and the effect of functional diversity is only tested on the residue. In this study, the effects resulting from both management and functional diversity are not assessed. Management and climate can explain a large variance of the functional diversity. The effect of functional diversity and management on ecosystem

services has to be studied conjointly, rather than separately or successively. Presently, evaluation of ecosystem services from management through functional diversity is still impossible. This study is a contribution to the current related research and cannot, at this time, be used by policy makers or farmers.

III. Addition of the inaccuracies for a future evaluation of ecosystem services

A. Addition of the inaccuracies

The objective of this PhD was not to propose indicators of ecosystem services from management and climatic data. However, the results of the various chapters may be useful for extrapolating the capacity of the current knowledge to evaluate the effect of management on ecosystem services. I evaluated the inaccuracies of some key points of the framework of ecosystem services assessment proposed by Lavorel *et al*. (2013). In the various chapters, I evaluated different errors: firstly, the confidence of the experts and literature on the links between functional diversity and ecosystem processes (Chapter 1), secondly, the capacity to calculate functional diversity from botanical surveys and functional trait databases (Chapter 2), and thirdly, the errors of the estimation of functional diversity from management and climate (chapter 3). An estimation of the accumulation of these different errors could be useful for extrapolating the capacity to produce indicators of the 8 ecosystem services using management and climate variables as inputs and functional diversity as an intermediate step.

From the results of these different estimations of errors, I developed decision trees to aggregate the different errors using DEXI software and proposed an evaluation of the error at the level of the ecosystem services. The first aggregation made was between the accuracy of the calculation of the functional diversity (see Table.2.12) and the percentage of variance explained by management and climate (see Table.3.13). The goals of this aggregation were: (i) to evaluate if the poor explanation of a functional diversity criterion from management and climate was due to the low accuracy of the calculation of the criterion from the functional trait database. For example, the functional dispersion of specific leaf area was poorly explained by management and climate, and was strongly influenced by the intraspecific variability of the functional traits and by the difference in sampling area. At the opposite, the community weighted mean value of the leaf dry matter content had an average estimation from the management and the climate (10.94%) and was very robust to the different errors tested in the chapter 2. The low explanation of CWMLDMC from the management and climate was not due to the problem of calculation of this criterion from a functional trait databases. (ii) The second goal of the aggregation was to eliminate some “false positive” relationships between functional diversity, climate, and management, i.e., a criteria well explained by management and climate, but strongly influenced by the different errors tested in the second chapter. For example, the functional evenness criteria were well explained by management and sufficiently by climate. However, functional evenness was very strongly influenced by difference in sampling area and deletion of minor species. Furthermore, the origin of the data (i.e., one of the three datasets) had a very strong impact on the functional evenness.

The aggregation between the accuracy of the calculation of the functional diversity and the percentage of variance explained by the management and the climate present a level of confidence in the estimation of each functional diversity criterion from management and climate. The level of accuracy was, subsequently, aggregated with the confidence of the expert on the connections between the processes and the criteria. The levels of accuracy of each process were obtained by these aggregations.

The levels of accuracy of the different processes were thus aggregated to propose a level of accuracy of the different functions and ecosystem services. For the biodiversity services, the percentages of aggregation proposed by the expert in the interview were used to build the decision rules aggregation. For the other services; each process had the same weight in the aggregation.

Table.D.1 Scoring (out of 5) of the accuracy of the different services regarding the errors at the different levels for different criteria and process

Services	Score
Bee	2/5
Bumblebee	1/5
Spiders	3/5
Orthoptera	3/5
Butterflies	3/5
Earthworms	1/5
Nitrogen Cycle	2/5
Water Cycle	3/5
Carbon Cycle	4/5
Biomass Yield	2/5
Stability	1/5
Forage Quality	1/5

The different scores of accumulation of errors for the different services are presented in Table D.1. The highest score, 4/5, was only observed for the carbon cycle service. This high accuracy was mainly due to the fact that the functional diversity criteria in the carbon cycle are well explained by management and climate. The strength of the links from the expert interviews in the carbon cycle service was generally well established. However, the mechanisms in carbon sequestration are complex, and, therefore, a proposition of indicators needs more work in order to be efficient. Medium accuracy (3/5) was found for 4 services (spiders, orthoptera, butterflies, and water cycle). For the water cycle, the medium accuracy could be explained by the poor confidence in the relationships between process and functional diversity. For the others, medium accuracy resulted, in part, from the low accuracy of the estimation from management and climate, and also from medium accuracy of the links between process and diversity. Three services had a low score (2/5); bee abundance, nitrogen cycle, and biomass yield. For bee conservation, the low accuracy was mainly due to the fact that pollen and nectar quantity are very poorly explained by management, which is an artifact of the high percentage of missing data for these traits. For the nitrogen fertility and biomass yield, the main contribution to the low score was the very low accuracy of estimating leaf dry matter content from management and climate. The restricted ability of management and climate to explain leaf dry matter content was not a result of the problem of extracting information from functional trait databases. This low value was difficult to explain given the results of other leaf economics spectrum traits, such as specific leaf area. Numerous studies have shown that leaf dry matter content was affected by climate, soil fertility, and disturbance (Garnier *et al.*, 2006; Ordoñez *et al.*, 2009; Michaud *et al.*, 2012b). Improvement to the estimation of leaf dry matter content would reduce the errors on the evaluation of these services.

The estimation of four different services had a very low quality (1/5). The very low accuracy of bumblebee conservation service was due to the low quality of the vegetation

structure variables (functional dispersion and mean vegetative height value). For the forage services (stability and forage quality), the very low accuracy originated from both the management estimation of the functional diversity criteria and from the low confidence of the experts.
These accumulations of errors demonstrate that the evaluation of ecosystem services from management and climate are not very accurate. Based on the results and conclusions of our various works, improvements to the framework can be proposed.

B. How to improve the evaluation of ecosystem services?

(i) A key point is to **propose clear definitions of ecosystem services /processes/ states,** and also to **propose standard measures of ecosystem services.** For example, forage services are sometimes described by standing biomass (Lavorel *et al.*, 2011), or annual biomass production (Baumont *et al.*, 2012). Standard measures of ecosystem services would be useful in comparing results between studies and to propose meta-analysis. Each definition and measure of ecosystem services chosen in this work could be challenged.
(ii) More research has to be done on the **connections between functional diversity and ecosystem functioning**. For some ecosystem services, the relationships have already been extensively studied (de Bello *et al.*, 2010). However, the observed relationships sometimes vary between studies. For example, functional identity was linked to aboveground biomass productivity in some studies (Lavorel *et al.*, 2011; Lavorel and Grigulis, 2012); however, other studies showed an effect of functional divergence (Mokany *et al.*, 2008; Klumpp and Soussana, 2009; Mouillot *et al.*, 2011) or functional regularity (Mouillot *et al.*, 2005; Mokany *et al.*, 2008) on biomass production. Also, the functional traits vary from study to study. In some studies, several functional traits were used (Mouillot *et al.*, 2005; Mokany *et al.*, 2008; Mouillot *et al.*, 2011), while other studies used only one (Klumpp and Soussana, 2009; Lavorel *et al.*, 2011; Lavorel and Grigulis, 2012). The functional traits used are sometimes the specific leaf area (Klumpp and Soussana, 2009), the vegetative height or the leaf nitrogen content (Lavorel and Grigulis, 2012). The theoretical understanding to unify and explained all of these results is still lacking. Results of the interviews with the experts also demonstrated a lack of information. Firstly, the links between "hard" traits and "soft" traits have to be further studied (Violle *et al.*, 2007). One example is the carbon allocation between leaf and root, for which the experts could only propose some hypotheses. Yet, this allocation is a key parameter in biogeochemical cycles. Other hard traits used in the model could also be related to soft traits, such as the timing of emergence of new leaves. Combining a modeling approach and functional diversity would improve the evaluation of ecosystem services. Furthermore, the position of functional diversity criteria regarding the ecosystem functioning and services should be clarified. Indeed the functional diversity could cause the ecosystem functioning or could results of the ecosystem functioning or could only be an indicator (i.e. influence by the same process).
(iii) The functional diversity criteria we used were the only available functional diversity criteria, but not necessarily the best ones to evaluate ecosystem services. Indeed, we occasionally used proxy, i.e., an available functional diversity criteria, instead of unavailable functional diversity criteria. For example, instead of root traits, we used leaf traits. The number of species was also often used to describe diversity of food sources. Some functional traits may be useful to evaluate this diversity of food sources (flower traits and leaves chemical characteristics). In this PhD, one main hypothesis was that functional diversity is a good predictor of ecosystem services. Some very recent studies focus on the interest of phylogenetic diversity (Cavender-Bares *et al.*, 2009). For instance, phylogenetic diversity was found to rely on the phylogenetic distance between species. It has also been proposed that phylogenetic diversity is a proxy of the overall functional diversity by

Cadotte *et al.*, (2013). Biomass quantity is related with the phylogenetic diversity (Cadotte *et al.*, 2011) and also with animals taxa (Dinnage *et al.*, 2012). The phylogenetic also respond to disturbance soil and climate (Bernard-Verdier *et al.*, 2013). Including phylogenetic diversity in this type of study could lead to interesting results. However, with phylogenetic diversity, the understandings of the links are less mechanistic. Testing the effect of management and climate on phylogenetic diversity would be interesting, as well as comparing functional diversity and phylogenetic diversity.

(iv)**The measurement of functional diversity should be improved**. Field measurements are, without question, the most accurate way to measure functional diversity. However, field measurements are very time consuming, especially in highly diverse ecosystems. Measurements generally only focus on the dominant species and few traits. These simplifications have consequences for certain components of functional diversity. More tests should be conducted in order to propose a simple and accurate protocol. The option is to measure intraspecific variability, which contributes information about the community assemblage (Jung *et al.*, 2011; Violle *et al.*, 2012) is another advantage of taking field measurements. Field protocols to measure intraspecific variability should be proposed by the research community (Violle *et al.*, 2012). Functional diversity, as in this study, can be estimated using species composition and functional traits from a database. Our work demonstrates many limitations in the use of functional trait databases, such as missing data. In the case of missing data, imputation methods can be useful to fill data gaps for the most used functional traits (see chapter 2 and annex III). However, for some traits, like roots and flower traits, the percentage of missing data is very high. Targeted field measurements on certain species and traits could be made in order to fill the functional trait databases. Intraspecific variability is also a notable problem in the use of functional trait databases. Understanding the pattern and the drivers of intraspecific variability are key points for improving the use of functional trait databases. This understanding can only be achieved by regrouping numerous datasets. For the pattern, examining relationships between species traits value and functional identity is a good way to evaluate the pattern of intraspecific variability (Ackerly and Cornwell, 2007). Evaluation of the drivers of intraspecific variability is also a key point necessary to select the most accurate value in the functional trait database for species with several values presents in the database. Some drivers of intraspecific variability have already been tested in the literature (Albert *et al.*, 2010a; Albert *et al.*, 2010b).

(v) Not all potentially important variables in the estimation of functional diversity criteria could be taken into account in this work. The **study of historical effects** could be also a key point for the estimation of functional diversity. The dynamics of flora has already been studied, in the case of extensification (Louault *et al.*, 2005; Fayolle, 2008), intensification (Laliberté *et al.*, 2012; Janeček *et al.*, 2013), and even without significant change of management (Peter *et al.*, 2008; Peter *et al.*, 2009). However, in these studies, the different sources of species (seed pool, landscape) that induced change in the flora were poorly described. Surveys of nutrient dynamics in the ecosystem during a change in management would also be helpful to fully understand the change (or absence of change) in the flora. A harmonization of the management data would be necessary to compare the different studies. Harmonization for the functional traits (Cornelissen *et al.*, 2003; Kattge *et al.*, 2011; Pérez-Harguindeguy *et al.*, 2013) and for soil (Bartholomeus *et al.*, 2008) have already been proposed. Large scale studies with complementary protocols also offer potential, but would require a lot of work (Garnier *et al.*, 2006; Bernhardt-Romermann *et al.*, 2011).

IV. Conclusions

During this PhD project, we used functional trait databases to assess functional diversity. This method, which has to be used when field measurements are not possible, induced notable errors when predicting functional diversity indexes, and we, therefore, proposed different methods to evaluate these errors. In the case of missing data, imputation methods can be used to replace missing data when up to 30% data is missing. We also proposed a framework to evaluate the effect of intraspecific variability.

Evaluation of some ecosystem services using plant functional diversity has been proposed and tested in many studies (Diaz and Cabido, 2001; Kremen, 2005; Diaz *et al.*, 2007a; de Bello *et al.*, 2010; Diaz *et al.*, 2011). The effects of management and climate on functional diversity have also been tested (de Bello *et al.*, 2005a; de Bello *et al.*, 2005b; Diaz *et al.*, 2007b; Flynn *et al.*, 2009; Bernhardt-Romermann *et al.*, 2011; Bernard-Verdier *et al.*, 2012). Logically, evaluation of the effect of management and climate through functional diversity was proposed by different authors (Garnier *et al.*, 2006; Lavorel and Grigulis, 2012; Lienin and Kleyer, 2012; Lavorel *et al.*, 2013). In this PhD project, management and climate were found to have a strong influence on some functional diversity criteria related to ecosystem services. Indeed, on a list of 29 functional diversity criteria selected for their relationships to 8 ecosystem services, 10 criteria were strongly related to management and climate variables, and 10 other criteria had an average link with management and climate. These strong and average links were based on conditional effects, in other words the management effects varied depending on the climatic conditions. Indicators of ecosystem services that take functional diversity into account are necessary for the evaluation of grasslands that differ in botanical composition. But, because the botanical composition of the grasslands might be unknown in a large number of studies, estimation of functional diversity using management and climate would be useful. These estimations of functional diversity could be used afterwards in ecosystem services evaluations tools.

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Annexes

Annex I: Progress report on the effect of grassland management on grassland biodiversity from the field to the landscape level as input for Task 3.4 and 3.5

A. Summary

Multi-species swards could be a key element for an improved delivery of provisioning, regulating and supporting services from grassland-based ruminant production systems. One of the objectives of the MULTISWARD project is to provide evaluation tools (indicators and models), sensitive to the effects of plant diversity on ecosystem services, to assess production systems based on the utilization of multi-species swards. But plant diversity is laborious to measure in the field and the plant species richness of agricultural grasslands has been shown to be strongly influenced by agricultural management. If strong relationships between agricultural management and plant diversity characteristics relevant to the delivery of ecosystem services can be found, then the development of management-based indirect indicators including the effects of plant diversity on the delivery of ecosystem services could be possible.

The aim of this report is to review current knowledge about the effects of agricultural management on the diversity of grassland communities, taking into account the influence of the pedo-climatic conditions, as an input for the development of indirect indicators of diversity planed in the MULTISWARD project. This deliverable focus on the effects of agricultural management on functional diversity because functional plant traits are of great importance for understanding the response of plant community structures to abiotic, biotic and anthropogenic factors, because such traits plays an important role in the delivery of ecosystem services (de Bello *et al.*, 2010b), and because the effects of agricultural grassland management on plant species richness has already been discussed in numerous reviews (e.g. Gaujour *et al.*, 2011; Dumont and Tallowin, 2011). To achieve this objective, a literature review encompassing agronomical as well as ecological studies on factors influencing grassland plant diversity was performed.

The MultiSward partners involved in the preparation of this deliverable are:

- the team of Nancy-Colmar of the Institut National de la Recherche Agronomique (INRA, Mixed Research Unit "Agronomie et Environnement"), and
- Agroscope Reckenholz-Tänikon Research Station ART (FDEA-ART).

After a short summary of the effects of agricultural management on plant species richness, this deliverable develops the currently known effects of management on the following functional traits of grassland communities: the Specific Leaf Area (SLA), the Leaf Dry Matter Content (LDMC), the Leaf Nitrogen Content (LNC), the Rooting Depth, the Flowering Date, the presence and the relative abundance of Legumes in the cover, and the presence and relative abundance of entomophilies species. It considers five different components of functional diversity at the community level and is completed by some considerations regarding plant diversity at the landscape level.

List of abbreviations

FI	Functional Identity
FR	Functional Richness or Amplitude
FE	Functional Evenness or Regularity
FV	Functional Divergence
FD	Functional Dispersion
SLA	Specific Leaf Area
LDMC	Leaf Dry Matter Content
LNC	Leaf Nitrogen Content
RD	Rooting Depth

B. Introduction

Biodiversity was defined during the Earth summit of Rio (1992) as the diversity of form of life. It is now decreasing under human pressure (Sala *et al*., 2000). In agricultural systems, this decrease has mainly been driven by agricultural intensification and simplification of the agri-ecosystems (e.g. Tallowin et al., 2005; Le Roux et al., 2008). But less biodiversity within an ecosystem can affect its functioning and lead to a reduced delivery of ecosystem services (see MultiSward deliverable D2.3-D3.1 for a review). A major challenge of agricultural production is therefore the maintenance or recovery of diverse agri-ecosystems to sustain the diverse ecosystem services provided by agricultural land. A better understanding of the effects of agricultural management on biodiversity loss and of the effects of this loss on the services of agri-ecosystems is thus needed.

One of the major goal of research in ecology since the beginning of last century is the understanding of the mechanisms driving biodiversity. How can species coexist within an ecosystem? Why do some ecosystems have more biodiversity than others? How can the presence/absence of a species inside a community be predicted? A lot of studies tried to answer one of these questions for different types of communities or ecosystems and several theories have been proposed to explain the dynamic of communities (e.g. Tilman, 1982, 1994; Hubbell 2001). Keddy (1992) developed a theory explaining the species assemblage of communities by the presence of ecological filters: species and individuals are filtered by the environmental conditions. These filters can be abiotic, like climate or soil conditions or biotic (trophic interaction or competition). The presence/absence of the species therefore depends on the traits of the species that determine the ability of the species to be competitive or not under the action of the ecological filters. Functional traits allow assessing the functioning of species (or individuals). A functional trait is any morphological, physiological or phenological feature measurable at the individual level, which impacts fitness indirectly via its effects on growth, reproduction and survival (Violle et al., 2007). Nutrient rich soil conditions usually favor few species having a strategy of resource capture, excluding more numerous species having a strategy of resource conservation (Grime, 1979; on the contrary to plant species with a resource capture strategy, species with a resource conservation strategy generally have slow growth rates, long lived leaves and high rates of nutrient retention). In contrast, in oligotrophic soil conditions only few species can survive, resulting in low species richness, maximal species richness being generally observed in mesotrophic conditions (Grime, 1979; Rajaniemi et al., 2002; Gross et al., 2005). Although the unimodal pattern of plant diversity along gradients of nutrient availability has recently been questioned (Adler et al., 2011), this shows that ecological filters might not only influence species assemblage, but also the plant species richness of a habitat. In agri-ecosystems, agricultural activities also act as ecological filters by modifying the environmental conditions in the system. In grasslands for instance, defoliation by mowing or grazing strongly modifies light availability and temperature at the soil surface. Fertilization and irrigation or drainage affect nutrients and

water availability, and therefore alleviate some stresses (a stress being here a factor that limits the growth of the plants (Grime, 1979). In agri-ecosystems, the anthropogenic filters therefore combine with the abiotic and the biotic filters to impact species assemblage and richness.

At community level, the assemblage of the functional trait values of the individuals (or species) composing the community is defined as functional diversity. Functional diversity is so a resultant of the effects of ecological filters on the community and its study allows understanding the assemblage community rules (McGill et al., 2006). Functional diversity can be described for both continuous functional traits (e.g. vegetative height of the individuals of the community) and discrete ones (e.g. photosynthetic pathways of the individuals of the community). It can simultaneously consider several traits, but the direct measurement of functional diversity is laborious. Different components of functional diversity have been described (Mason et al., 2005): functional Identity, functional richness (or amplitude), functional evenness (or regularity), functional divergence and functional dispersion (Figure 1 and Box 1). Functional diversity indexes are calculation methods allowing quantifying one of the functional diversity components. They are based on the functional traits values of the species/individuals of the community and, for some of them, on the relative abundances of the species in the community (Schleuter et al., 2010). Functional diversity is a key parameter to link ecosystem functioning to the biodiversity of communities (de Bello et al., 2010b).

Because of the importance of functional plant traits for understanding plant community structures and compositions, because functional plant traits influences the delivery of ecosystem services (de Bello et al., 2010b), and because the effects of agricultural grassland management on plant species richness has been discussed in numerous reviews (e.g. Gaujour et al., 2011; Dumont and Tallowin, 2011), we focus here on the effects of agricultural management on functional diversity. The aim of this report is to review current knowledge about the effects of agricultural management on the functional diversity of grassland communities, taking into account the influence of the pedoclimatic conditions, as an input for the development of indirect indicators of functional diversity planed in the MultiSward project. Nevertheless, current knowledge about the quantitative effects of agricultural management on the trait values of grassland communities is in general poor because these effects are confounded by the effects of the environmental conditions. The database created in MultiSward will serve to better characterize these effects for European grasslands.

C. Short summary of the effects of agricultural management on plant specific richness

A recent literature review identified the factors affecting plant biodiversity in managed grasslands with a focus on specific richness (Gaujour et al., 2011). Management intensity, i.e. the frequency of defoliation and the concomitant increase in fertilizer application, strongly influence the number of plant species composing grassland communities (e.g. Kleijn et al., 2009). The number of cuts per year and the earliness of the first cut during the growing season affect plant species richness (e.g. Zechmeister et al., 2003). These two factors are usually interdependent. Specific richness was found to be lower in abandoned grasslands than in grasslands being managed at a low level of disturbance (Bakker and Berendse, 1999). A further increase in the frequency of defoliation reduces the number of species. The date of first exploitation is a key element: an early cut excludes non-clonal species and a late cut results in dense biomass unsuitable for the settlement of new species (Smith et al., 2002; Barbaro et al., 2004). The maximum specific diversity is generally obtained by quite late harvest dates: from mid-June to mid-July in European zones with oceanic or semi-continental climates (Critchley et al., 2007).

Most plot experiments with fertilization on permanent grasslands show that increasing applications of mineral or organic fertilizer result in a drop in plant species richness and changes in botanical composition (e.g. Schellberg et al., 1999). These trends, frequently observed in mid-term experiments, are confirmed both in long term experiments (Silvertown et al., 2006), and in gradients of soil fertility on farmland (Isselstein et al., 2005). The study of Kirkham et al. (2008) suggests that at similar levels of N, P and K applications, mineral fertilization is no more detrimental to species richness than farmyard manure. But as discussed by the authors, the amount of nutrient applied in the organic fertilizer treatments might in fact have been higher than in the inorganic treatments. Numerous authors have demonstrated that N influences botanical composition and species richness from an input of 30 kg N ha-1 yr-1 (e.g. Joyce, 2001; Mountford et al., 1996; Plantureux, 1996). This threshold is lower if high patrimonial value species are considered, because of their high sensibility to soil nutrient level (Grevilliot et al., 1998). Fewer studies focused on the influence of P and K on biodiversity, but most authors like Janssens et al. (1998) showed negative effects of soil P and K fertility on plant species diversity. Several works came to the conclusion that P is a key factor for plant diversity (e.g. Ceulemans et al., 2011).
Compared to mowing, the influence of grazing on plant species richness can be positive, negative or neutral (Olff and Richie, 1998; Marriott et al. 2004; Fischer and Wipf 2002). A general trend is thus difficult to draw. Cingolani et al. (2005) suggested that, for productive grazed grasslands, plant diversity shows a unimodal pattern, with low biodiversity in almost abandoned and intensively grazed pastures. The primary role of grazing animals for biodiversity is maintenance and enhancement of sward structural heterogeneity by selective defoliation, treading, nutrient cycling and propagule dispersal. At the same time grazing animals maintain high selection pressure on species (Rook and Tallowin, 2003). While mineral fertilizers increase the homogeneity of soil fertility, nutrients deposited by grazing animals can provide heterogeneity beneficial to biodiversity. In most cases however, the mean plot increase in nutrient fertility offsets the heterogeneity, and plant diversity thus decreases (Reynolds et al., 2007).

D. The components of functional diversity and their indexes

Functional identity (FI): corresponds to the mean value of the functional trait of the individuals of the community. This value is a way to sum up all trait values by creating an average individual of the community. Community mean value or aggregated trait is an index able to evaluate the functional identity of a community (Grime, 1998; Lavorel et al., 2011; see Figure 1 for a graphic presentation of the different components of functional diversity).
Functional Richness or Amplitude (FR) corresponds to the range of the values taken by the functional trait of the individuals of the community. When the trait is continuous, functional richness is the amplitude of this functional trait. When the trait is discrete, functional richness is the number of modality of the functional characteristic (i.e. number of groups). Functional richness can also be evaluated on several functional traits. Functional richness corresponds to the functional space occupied by the community and it allows evaluating its functional ecological niche. Ecological niche results of a succession of ecological filters. Several functional richness indexes using functional trait exist. Some are based on the amplitude or space of one or several traits: Functional range (Mason et al., 2005), convex volume (Cornwell et al., 2006) or FRis index (Schleuter et al., 2010). Others indexes are based on the calculation of distances: FAD (Walker et al., 1999), FD (Petchey and Gaston, 2002) modified by Mouchet et al., (2008) and the number of group (Petchey and Gaston, 2006).

Functional Evenness or Regularity (FE) corresponds to the repartition of the abundances inside the functional space (Mason et al., 2005; Mouillot et al., 2005; Villeger et al., 2008). The more uniformly distributed the abundance between the different values of the functional trait is, the higher is the evenness. The evenness allows studying the biomass/ abundance distribution inside the functional space. If the value of the functional trait does not affect the abundance of the species in the community and the abundance is equally distributed between the different values of the functional trait, evenness will be high. High regularity can be the result of competition. Some Hypothesis in community ecology supposed that in the same community two individuals can not have the same value of a functional trait. This is the limiting similarity hypothesis. By the effect of competition, the abundance is homogenous distributed along the functional space. Evenness can be measured by the Shannon weaver index when the functional trait is discrete. For continuous trait, the FROm index (Mouillot et al., 2005) can be used for one trait and the FEve index for multi trait approach (Villeger et al., 2008).

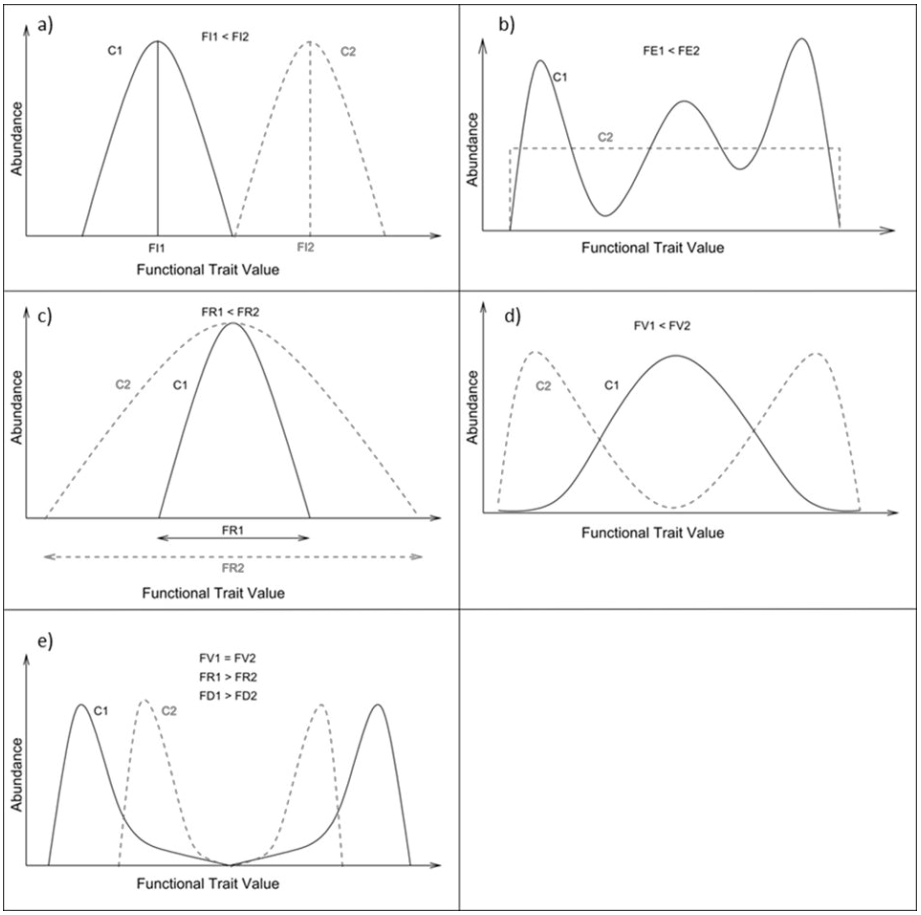

Figure 1: Different components of functional diversity. The x-axe represents the value of one functional trait; the y-axe represents the abundance of the different species inside the community. For each component, two example communities (C1 and C2) with opposite functional diversity situation are represented. a) Functional identity (FI) can be defined as the community average value of a functional trait; b) Functional evenness (FE) corresponds to how regularly species abundances are distributed in the functional space; c) Functional richness represents the amount of functional space occupied by a species assemblage; d) Functional divergence defines how far high species abundances are from the center of the functional space; e) Functional dispersion is a mix of functional richness and functional divergence;

Functional Divergence (FV) corresponds to the repartition of the abundance regarding functional identity (Mason et al., 2005; Villeger et al., 2008; Mouchet et al., 2010). When the abundance is on the extreme values of the functional space, functional divergence is high. At the opposite when most of the abundance is concentrated around the mean value,

the functional divergence is low. Functional Divergence allows identifying the abundance rules inside the communities and the number of functional strategy. When only one functional strategy is dominant, the divergence is low. When at least two strategies are dominant, the divergence is higher. Divergence results of the functional strategies responses to the environmental filters. When a filter create a functional convergence (selection of individuals with the same functional characteristic), the divergence is low. But species can respond very differently to an environmental factor. These differences create a high divergence. For the divergence of one trait, two indexes can be used, the mason index (Mason et al., 2003) and the FDis index (Schleuter et al., 2010). For the calculation of the divergence with several traits, only the FDiv (Villeger et al., 2008) index is available.
Functional Dispersion (FD) is a very new concept (Laliberté and Legendre, 2010). This is a combination of functional amplitude and functional divergence. The Rao index and the FDisp index allows to calculated Functional dispersion.

E. Choice of functional traits for this study

The following functional traits were selected for this study on the effects of management on functional diversity: the Specific Leaf Area (SLA), the Leaf Dry Matter Content (LDMC), the Leaf Nitrogen Content (LNC), the Rooting Depth (RD), the Flowering Date, the relative abundance of legumes in the cover, and the relative abundance of entomophilous species. These traits were chosen based on their relevance in the discussion around the delivery of ecosystem services and the availability of information in the literature.
The SLA is the leaf area divided by the dry mass of the leaf and help describing the growth strategy of the plants. The LDMC is defined as the leaf dry mass divided by leaf fresh mass. The LNC is the quantity of nitrogen in the leaf divided by the leaf dry mass. The RD evaluates how deep the roots colonize the soil. The Flowering Date is a good indicator of the phenology of the species/individuals. The relative abundance of Legumes in the cover is important for the nutrient dynamics. The relative abundance of entomophilous plant species is important in the assessment of the quality of the cover for pollinators.

F. Specific leaf area (SLA)

1. Effects of agricultural management on specific leaf area at individual scale

The SLA, defined as the leaf area per unit of leaf dry mass, is one of the key functional traits. It can be used to define the “Grime-strategy” of the species (Westoby, 1998). The SLA is positively linked to the photosynthetic capacity of the plant (Wright et al., 2006). A species with a high SLA thus have a high photosynthetic capacity and so generally a rapid growth rate. The SLA can also be linked to some ecosystem function like the vegetative biomass accumulation and the transpiration (Mokany et al., 2008). Table 1 shows values of the SLA for a few common temperate grassland species to illustrate the variability found in this trait.
The SLA is highly affected by the water balance of the ecosystem (Wright et al., 2001; Wright et al., 2004; Wright et al., 2005). Plant species with a high SLA cannot survive under dry conditions. A high SLA induces a large area of transpiration for the plant, unsuitable under water stress conditions (Wright et al., 2001; Ackerly and Cornwell, 2007). Species with a low SLA are more drought resistant. On the other hand, if species with a high SLA are not filtered by water stress, these species may outcompete species with a low SLA. The water balance of the ecosystem can be modified by irrigation or drainage.
The SLA is also affected by nutrient availability (Aerts and Chapin Iii, 1999; Ordonez et al., 2009). High SLA species have a quick nutrient turnover (quick nutrient acquisition and high nutrient losses), while low SLA species have a low nutrient acquisition but efficiently retain the acquired nutrients (Wright et al., 2004). In oligotrophic grasslands, species with a high

SLA cannot acquire enough nutrients to survive. Under eutrophic conditions, high SLA species thrive and outcompete low SLA species. Nutrient availability is affected by the fertilization practices. With mineral fertilization the nutrients are rapidly available to the plants, while with organic fertilization a large proportion of the nutrient first need to be mineralized.

Table.1. SLA, LDMC and Flowering date trait values for a few common temperate grassland species as an example. The values for the SLA and the LDMC were extracted from the LEDA trait database (www.leda-traitbase.org) and the flowering date adapted from the e-FLORA-sys database (http://eflorasys.inpl-nancy.fr).

species	**SLA in mm2/mg**	**LDMC in mg/g**	**Flowering date in day of the year**
Lolium perenne	25.67	214.4	160
Phleum pratense	23.21	276.1	190
Alopecurus pratensis	17.96	308.1	140
Bromus errectus	17.03	339.6	160
Nardus stricta	9.44	474	140
Trifolium pratense	23.37	223.4	150
Trifolium repens	31.44	185.7	160
Onobrychis viciifolia	18.01	255.9	160
Cichorium intybus	17.1	136	245

The frequency of defoliation also affects the SLA identity of the community. Frequent defoliations filter the species with a high SLA having a rapid growth that allows the species to produce new leaves shortly after being defoliated. The species with a low SLA are unable to survive in frequently defoliated grasslands. Indeed these species cannot quickly replace the leaf losses by new leaves. These species are thus progressively eliminated. Extensification can be considered as a plant succession. During a plant succession, high SLA species are progressively replaced by low SLA species (Garnier et al., 2004). By the first step of the succession, high SLA species deplete the nutrients available in the soil. With the reduction in nutrient availability, the high SLA species become limited in their growth and the low SLA species can establish in the community. Grazing involves the selective defoliation of some of the species of the community by the grazing animals, while other species are not consumed (Diaz and Cabido, 2001; Diaz et al., 2007; Fayolle, 2008). These species are sometimes called “species resistant to grazing” (Fayolle, 2008). These species area characterize by a lot of plant defenses (chemical and physical ones). The SLA of these species is generally low. For the other species, only the species able to quickly regrowth after the disturbance survive inside the pasture (species tolerant to grazing; Fayolle, 2008). These species are characterized by a high SLA. Defoliation by the animals is not the only effect of grazing. It also induces disturbances by trampling and animal dejections. Animal dejections induce a heterogeneous increase in nutrient availability. At high stocking rates, animals trampling has a strong effect on the vegetation. Trampling is an unselective disturbance but its effects are generally heterogeneously distributed. These different effects are presented in Figure 2.

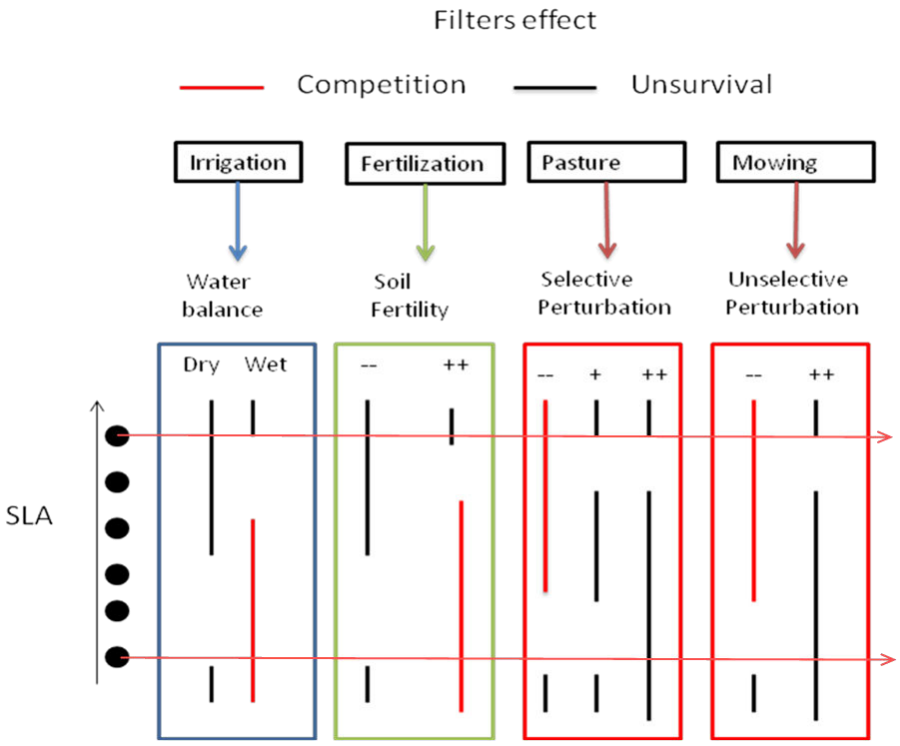

Figure 2: Effects of ecological filters on the SLA of species. The vertical axe of the figure represents the trait values (increasing trait value from bottom to top). Each box represents an environmental factors that affected the functional trait (in blue the environmental factors linked to the climate, in green the environmental factors linked to fertility, in red, the environmental factors linked to the perturbation of the grassland). Inside each box, the condition of the factor is represented by -- and ++ (for instance -- for low soil fertility and ++ for high soil fertility), and the effects of the factor on the selection of trait values is represented by vertical lines showing the different trait values eliminated by the filters. The filters can directly affect the presence/absence of species with trait values in a range (species with trait values in this range cannot survive under these filters). This type of effects is represented by a black line. Ecological filters often indirectly affect the range of the trait values of the species present in the community (species with trait values in this range could survive under this filter but are eliminated by competition). This type of effects is represented by a red line. The two horizontal red arrows represent the processes of trait selection. Where the arrow does not cross a vertical line, the trait value is present in the community. Where it does, the trait value is filtered and absent of the community.

2. Effects of agricultural management on the specific leaf area at community level

a. Community mean SLA value

Considering the effects of the different factors at the species level explained above, the community mean SLA value is expected to be high in nutrient rich, humid grasslands. On the contrary, the aggregated SLA value is expected to be low under nutrient poor, dry conditions (de Bello et al., 2005; Louault et al., 2005; Garnier et al., 2006). In frequently defoliated grasslands, the aggregated SLA is always high. Under nutrient poor, dry conditions, no grassland community can support a high defoliation frequency. When the disturbances are rare, the community mean SLA value mostly depends on nutrient availability and humidity: under eutophic conditions, the aggregated SLA remains high; under mesotrophic and oligotrophic conditions, the aggregated SLA is low. For pastures without fertilization, grazing seems to favor an increase in SLA (Fayolle, 2008). The species tolerant to grazing are the most dominant species in pastures. The species resistant to grazing are presents but their abundance is low (Fayolle, 2008). The ratio between tolerant and resistance species depends on the soil conditions and the climate (Pakeman, 2004; Vesk et al., 2004; de Bello et al., 2005, 2006).

b. Functional richness of SLA

Under grazing, functional richness is expected to be high, as two strategies of survival cohabit in the community (Fayolle, 2008), especially when nutrient availability and humidity are low/medium (de Bello et al., 2006).

c. Functional divergence and dispersion of SLA

Fayolle (2008) showed that abandoned pastures, unfertilized pastures, as well as fertilized pastures have a low SLA divergence, the relative abundance of the grazing resistant species being very low. Divergence is thus low, despite of the presence of two survival strategies. De Bello et al. (2006) showed for other traits linked to the response to grazing that the functional divergence under grazing is linked to the climatic conditions: divergence seems larger under dry than under wet conditions.

G. Leaf Dry Matter Content (LDMC)

The Leaf Dry Matter Content (LDMC) is obtained by dividing the dry mass of the leaf by the fresh mass of the leaf. The LDMC is negatively linked to the SLA. The LDMC nevertheless gives more information on the nutrient balance and the allocation of carbon inside the leaf than the SLA does. The study of the two traits allows a better assessment of the trade-off between the resources capture strategy and the resources conservation strategy. A high LDMC can be associated to a strategy of nutrient conservation.

1. Effects of agricultural management on leaf dry matter content at individual scale

The LDMC is mostly affected by the same filters as the SLA (figure 3). Water availability strongly filters the LDMC values of the plant species, because only species with a low LDMC are adapted to dry conditions (Wright et al., 2004; Michaud et al., 2012). The LDMC is also affected by nutrient availability (Ordonez et al., 2009; Ostonen et al., 2007), being low in nutrient rich grasslands and high in nutrient poor grasslands. Moreover, Michaud et al. (2011) showed that the LDMC is negatively affected by the quantity of solar radiation, being low under high solar radiation.
The LDMC of the species decreases with an increasing level of disturbance (Fayolle, 2008; Michaud et al., 2012). Species with a high LDMC lose too much biomass and nutrients when defoliated and are not able to replace them. On the other hand, when the frequency of defoliation is low, species with a high LDMC are strong competitors (Garnier et al., 2004). Under grazing, two different LDMC responses are possible, as for the SLA. Low LDMC characterizes species tolerant to grazing and able to re-grow after grazing (Fayolle, 2008). A high LDMC can sometime be related to the presence of physical or chemical plant protection (like the presence of condensed tannins). Such high LDMC species have a low palatability. By defoliating only some species of the cover, grazing animals induce a functional divergence of the LDMC.

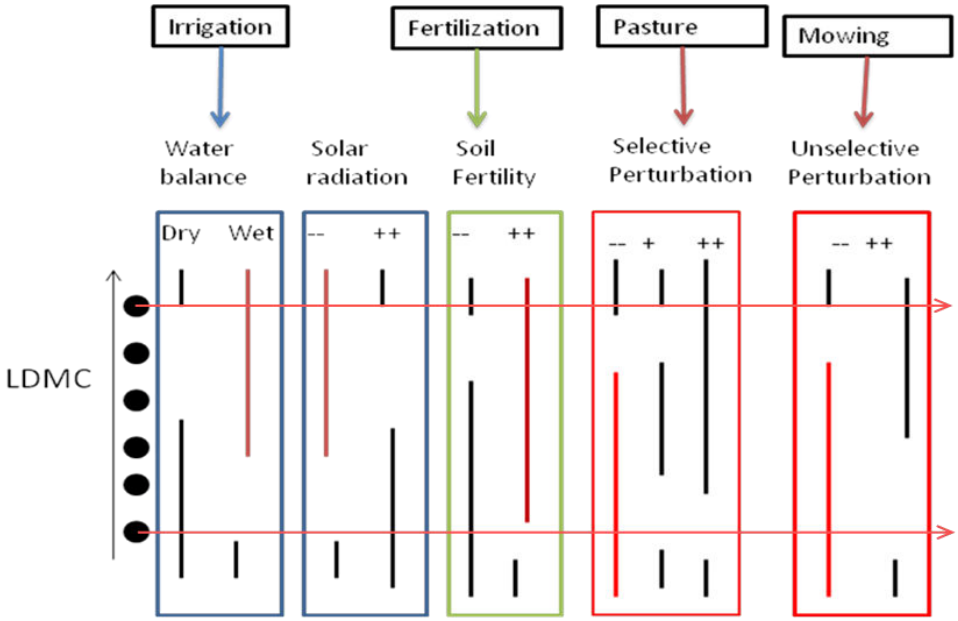

Figure 3: Effects of ecological filters on the LDMC of species. See legend of figure 2 for explanations.

2. Effects of agricultural management on leaf dry matter content at community level

a. Community weighted mean value LDMC

From the filtering of the LDMC values at the individual scale described above, the community mean LDMC value is expected to decrease with an intensification of agricultural management (increase in fertilization and in the frequency of defoliation; Fayolle 2008). Michaud et al. (2011) showed on 200 grasslands with contrasted management that the aggregated value of LDMC is driven by nutrient availability and by the level of solar radiation.

6.2.2. Functional richness of LDMC

Fayolle (2008) showed a decreased of the LDMC functional richness with increasing intensification (grazing, fertilization). But the author pointed out that this increase is due to the presence of only a few species with a very high LDMC. These results suggest that low LDMC species can survived in communities that are not frequently disturbed.

b. Functional divergence of LDMC

The divergence is higher in less disturbed grassland due to the presence of two survival strategies, but some other work shows a high divergence of LDMC for medium nutrient availability and medium level of disturbance (Duru et al., 2009; Duru et al., 2012). The climatic factors seem to be very important to explain these differences.

c. Functional regularity of LDMC

No information on the functional regularity of the LDMC of grassland communities was found in the literature.

H. Leaf nitrogen content (LNC)

The LNC is linked to the quantity of protein in the leaf, especially the RUBISCO enzyme. The LNC is therefore linked to the photosynthetic capacity of the leaf, and inform about the resource use strategy of the plant (capture or conservation of resources). It is linked to the

SLA and negatively correlated with the LDMC. Legumes usually have a higher LNC than other plant groups, thanks to their ability to symbiotically fixed atmospheric nitrogen.

1. Effects of agricultural management on leaf nitrogen content at individual scale

The LNC is mostly driven by nutrient availability, especially by the level of nitrogen available in the soil. But the LNC is also driven by phosphorus availability (Ordonez et al., 2009; Lavorel et al., 2011). The LNC is further an indicator of the growing conditions encountered by the plants (mean temperature and water balance; Ordonez et al., 2009; Lavorel et al., 2011). Under productive conditions (wet, warm and nutrient rich), species have a quick growth associated with a strong photosynthetic activity and so a high LNC. In less productive (dry and/or cold) grasslands, plant growth is limited, so the photosynthetic activity is lower and part of the nitrogen taken up by the plants is not allocated to the leaves (figure 4).

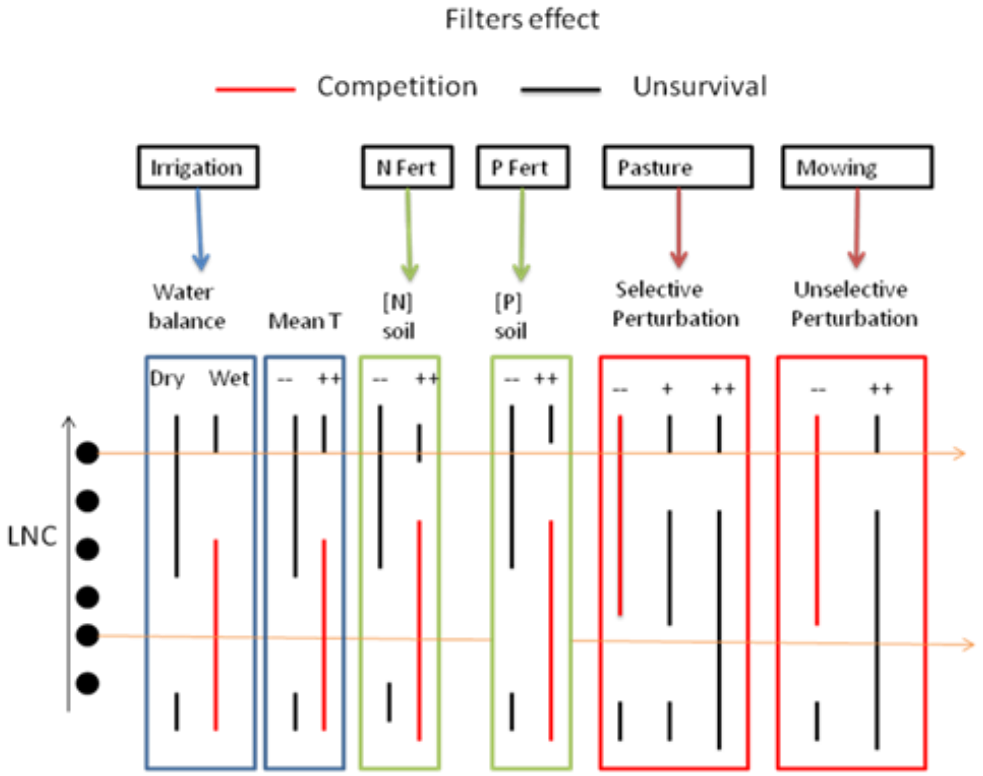

Figure 4: Effects of ecological filters on the LNC of species. See legend of figure 2 for explanations.

2. Effects of agricultural management on the leaf nitrogen content at community level

a. Community weighted mean value LNC

From the above considerations at the individual scale, it follows that the community mean LNC value is expected to be higher in productive grasslands. Lavorel et al. (2011) showed that the LNC value of the communities is driven by the nitrogen and the phosphorus availability. Nevertheless, because legume species usually have a high LNC and their relative abundance in the community can be promoted by low nitrogen fertilization, the relationship between nitrogen fertilization and the community mean LNC value might be not very tight for agricultural grasslands containing legumes. For instance, from the data of Nyfeler et al. (2011), the N concentration in the pure grass swards increased from around 15 g N kg-1 DW when N fertilization was increased from 50 to 450 kg N ha-1 yr-1, while it increased from only around 3 g N kg-1 DW in the grass-legume mixtures sown with 50% legumes (average of the second year after establishment; relative abundance of legumes

of approximately 50 and 20% for a fertilization of 50 and 450 kg ha-1 yr-1 respectively). In the study by Lavorel et al. (2011), the aggregated LNC value was also influenced by the type of land use and the altitude. Fayolle (2008) showed that abandoned grasslands have a lower LNC than grazed pastures. These differences can be due to the increased nutrient availability due to the animal dejections in the grazed pastures, or by an effect of disturbance. We can suppose that the LNC will react the same way as the SLA, and that the aggregated LNC value will be high under frequent defoliation and low at low level of disturbance. We can also suppose that selective defoliation by grazing can promote alternative strategies, high LNC species probably being tolerant to grazing and low LNC species being avoided by the grazing animals.

b. Functional richness of LNC

LNC functional richness is higher in grazed pastures than in abandoned grasslands (Fayolle, 2008). This could be due to firstly the filtering of two different strategies by the pressure of grazing (tolerant and resistant species), and secondly to an increase in the heterogeneity of nutrient availability due to animal dejections.

c. Functional divergence and evenness of LNC

No study showed an effect of agricultural management or climate on the LNC functional divergence (Fayolle, 2008; Lavorel et al., 2011). The evenness of LNC was, as far as we know, never study.

I. Flowering date

The flowering date is the date of the year when the flower appears. It can also be expressed as the sum of temperature at the time of the apparition of the flower. The flowering date is related to the phenology of the plant: species with a quick growth usually also have an early flowering.

1. Effects of agricultural management on the flowering date at individual scale

The flowering date described as the Julian day is mostly drive by the sum of temperature. Within a species, the relationship between the Julian day and the sum of temperature is very good and therefore some authors use the sum of temperature rather than the Julian date to describe the flowering date (Michaud et al., 2012). The effect of the sum of temperature is more important on forbs species than on grass species (Fitter and Fitter, 2002).
The date of flowering is filtered by the climate: climatic zones with summer dryness filter for earlier flowering than wet climates. The early flowering allows finishing the reproductive cycle while water availability is sufficient for seed formation. One alternative strategy under dry conditions is a slow growth with the possibility of entering dormancy during dry periods. In this strategy, flowering is usually triggered by the return of the rain in autumn. Species growing under oceanic conditions (mild winters) have a later flowering date that the one growing under more continental conditions.
Flowering time is also driven by agricultural management. In nutrient poor grasslands, the flowering date is usually later than in nutrient rich grasslands. That can be explained by the quick growth in the nutrient rich grasslands. The date of the first defoliation (mowing or pasture) and the number of yearly defoliations also influence the flowering strategy of the plants of the community. With an early first defoliation, more species with an early flowering date are usually present, because this gives them more chance to complete their reproductive cycle before being cut or eaten (figure 5).

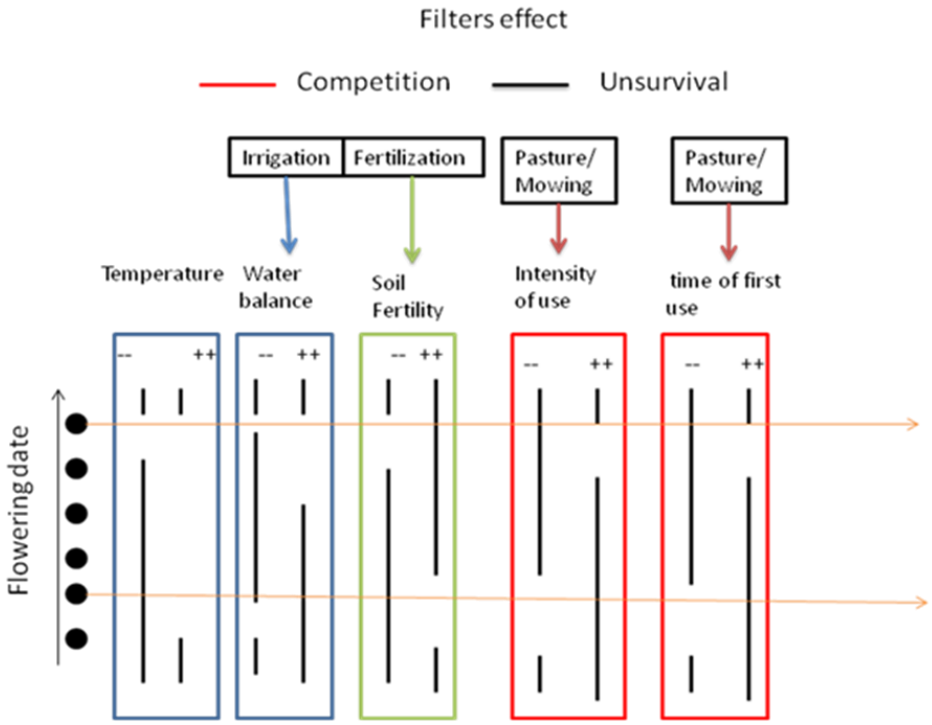

Figure 5: Effects of ecological filters on the flowering date of species. See legend of figure 2 for explanations.

2. Effects of agricultural management on the flowering date at community level

a. Community mean of the flowering date value

Along a large climatic gradient, the community mean flowering date value, as the sum of temperature, is driven by climatic factors related to water availability, by the oceanic–continental gradient, as well as by agricultural management. Intensively managed grasslands have earlier flowering date values as extensively managed ones (higher nutrient availability and earlier first defoliation). Within regions of similar climatic conditions, the flowering date is mostly driven by nutrient availability (Fayolle, 2008; Lavorel et al., 2011).

b. Functional richness, divergence and evenness of the flowering date

At the local scale, the functional divergence of the flowering date is low. The ecological filters have a strong effect on the flowering date creating a strong convergence. Lavorel et al. (2011) showed an effect of altitude on the divergence of the flowering date.
The functional evenness and richness of the flowering date have not yet been studied.

J. Rooting depth (RD)

Roots functional traits are very important to understand the uptake of nutrients and water in the soil and so the underground competition (Casper and Jackson, 1997). One of the hypotheses of the MULTISWARD common experiment is that diversity in term of root functional traits induces a complementary use of the available soil resources, thereof improving the functioning of the ecosystem and primary productivity. Several root traits influence nutrient uptake. The root surface is related to the exchange capacity between the soil and the plants. The root length is related to the capacity of the species to explore the soil. The rooting density of a species corresponds to its capacity to use a portion of the soil. The specific root length (root length/root mass) and the root dry matter content

characterize the economics of the roots (Ostonen et al., 2007). Roots architecture is another key parameter to understand underground functioning.
However root traits are very laborious to measure, especially in species rich communities. Moreover, their variability within a species is large. Therefore, roots traits are generally studied for single species and under control conditions. Mokany and Ash (2008) argued that such measurement might not be representative for plants growing in a community on a natural soil. The number of studies that assessed root traits on multi-specific grasslands is very low and only the rooting depth is documented enough for our study.

1. Effects of agricultural management on rooting depth at individual scale

First, rooting depth is driven by the available soil depth, the roots being only able to express their availability to colonized deep soil layers when the soil is deep (figure 6).
Limited nutrient and/or water availability filter for deeper rooting, because deep rooting allows the plants to explore a larger soil compartment. Under high nutrient and water availability, roots were shown to grow horizontally at high density rather than to explore deep soil layers (Schenk et al., 1999; O'Brien et al., 2007). If nutrients and/or water are poorly available, the plants develop longer, deeper root systems to improve the exploration of the soil. However in nutrient poor grassland, plant growth is slow so roots growth is slow

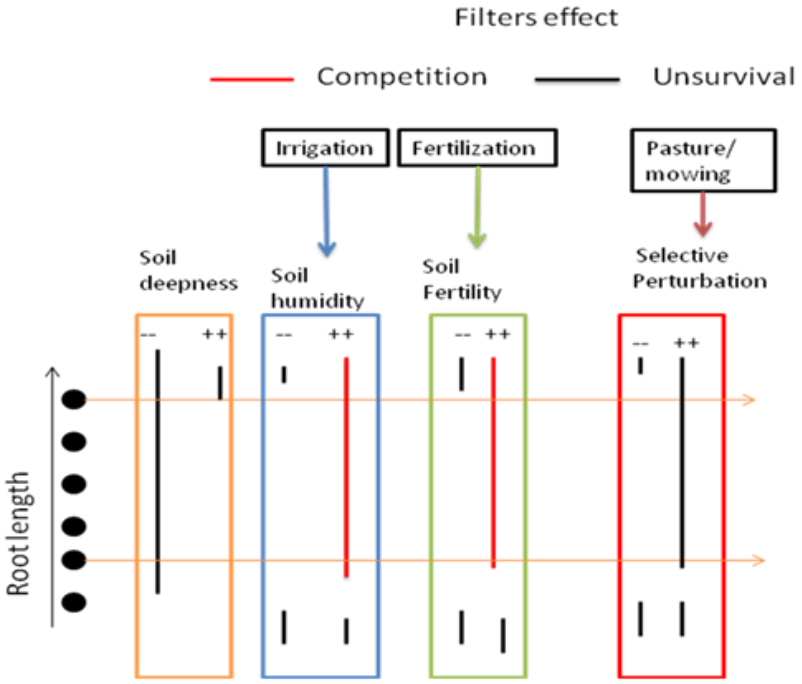

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Figure 6: Effects of ecological filters on the rooting depth of species. For explanations see Figure 2

The effect of disturbance on rooting depth is more difficult to assess. Defoliation affects root elongation through its effect on plant growth (Gastal et al., 2010). The response of rooting depth to defoliation seems to differ between species. The rooting depth of grazing **tolerant** species is for instance less affected by grazing than the one of species sensitive to grazing (Arredondo and Johnson, 1999; Gastal et al., 2010). Grazing also induces trampling that affects soil compaction and structure. Kumar et al. (2010) showed some differences between continuous and rotational grazing systems, with a higher root length density in the rotational grazing system.

2. Effects of agricultural management on rooting depth at community scale

No study at the community level was found. We can only suppose that the functional amplitude of rooting depth will be higher on nutrient poor grasslands with a low level of disturbance than on nutrient rich, frequently disturbed ones. This might be similar for the functional evenness of rooting depth.

K. Relative abundance of legumes

The species of the legume family (Fabaceae) are characterized by their availability to symbiotically fixe atmospheric nitrogen, which give them access to a nitrogen source that is unlimited at the plant scale. They therefore play an important role in the nitrogen cycle of the ecosystem, thereof influencing the functioning of the ecosystem and the production of biomass. The relative abundance of legumes impacts on the LNC value of the community and grasslands with high legume content may also be interesting for pollinator insects (Pywell et al., 2011). The relative abundance of legumes therefore interacts with other traits considered in this study.

Because of their ability to symbiotically fixe atmospheric nitrogen, legume plants have a competitive advantage under low N availability, but might not be strong competitors under conditions of high N availability (Houlton et al., 2008). Therefore, although the biomass production of pure legume swards is not influenced by nitrogen fertilization, nitrogen fertilization usually reduces the relative abundance of legumes in multi-species grassland communities (e.g. Nyfeler et al., 2009). Because phosphorus plays an important role in the functioning of the fixing root nodules (Israel, 1987), situations of P deficiency are unfavorable to high relative legume abundance. In a fertilization experiment on permanent grassland, high relative abundance of legumes was favored by phosphorus fertilization in the treatments receiving no or only few nitrogen (Huguenin-Elie et al., 2006).

Defoliation frequency also strongly influences the relative abundance of legumes in the community, with large differences in reaction between different legume species (Black et al., 2009). Species with an erect growth, like red clover and alfalfa, successfully compete with the grasses under lenient defoliation frequency. Species showing a prostrate growth structure, like white clover, profit from frequent defoliations because this increases light availability near to the soil surface and weaken the tall species competing with the legumes in the community. In frequently defoliated communities (5 cuts per year), white clover in completion with *Lolium perenne* and *Dactylis glomerata* was able to maintain a population over three years, even at very high level of nitrogen fertilization (450 kg $ha^{-1}yr^{-1}$; Nyfeler et al. 2009). It was also observed that white clover is able to increase its relative abundance even under high N fertilization, when the height of the community is maintained short by frequent grazing by horses. Díaz et al. (2007) analyzed 197 studies conducted worldwide and conclude that grazing favors small plants, low plants (vs erected plants) and stoloniferous plants, which fit with the above mentioned observations concerning white clover and high grazing pressure. Beside le frequency of defoliation, the date of the last defoliation of the year might also impact the relative abundance of white clover in grassland communities, as experimental results showed that leaf area of white clover during winter plays a crucial role for it survival during winter and growth speed in spring (Lüscher et al., 2001).

Lucero et al. (1999) showed that severe water stress might provoke a competitive disadvantage for white clover growing in association with *Lolium perenne*. Irrigation might on the other hand in the long term decrease the relative abundance of legume specie with a deep root system like alfalfa by favoring species less tolerant to dry condition.

L. Relative abundance of entomophilous species

Dry grasslands have more entomophilous species than wet grasslands (Michaud et al., 2012), and thus the relative abundance of entomophilous species is negatively linked to soil humidity. The effect of increasing the number of defoliations on the relative abundance of entomophilous species strongly depends on the starting point (defoliation frequency and botanical composition). Increasing the defoliation frequency on an extensively used, flower-rich grassland tends to reduce the relative abundance of entomophilous species. On the other hand increasing the defoliation frequency on an intensively used, grass-rich grassland even further, often decreases the relative abundance of grasses in favor of entomophilous species. Nevertheless, in this later case, very frequent perturbations and removal of the flowers might not be conductive to high insect abundance. The effect of grazing intensity depends on the feeding behavior of the animal species grazed. The percentage of entomophilous species is also driven by fertilization. Grass normally responds well to nitrogen mineral fertilization, decreasing thereof the relative abundance of entomophilous species. In some cases, fertilization might favor forbs species, increasing the abundance of entomphilous species (Michaud et al., 2012). This has often been observed under climatic conditions unfavorable to very productive grass species, like rye-grasses.

M. Diversity at Landscape Level

Landscape is defined as a level of ecological system organization above the ecosystem. It is characterized by heterogeneity and by dynamics that are partly governed by human activities. In agricultural systems, landscape can be described by the spatial distribution and the occupation of the different plots of the farm(s) and by the presences of non-agricultural elements (hedge, forest..) and of buildings. Two important questions are asked at this scale. How does the structure of the landscape affect the diversity at plot level? And, how to evaluate the diversity at the landscape level?

The landscape structure can be an explicative factor for species richness, landscape heterogeneity increasing the potential for high species richness at the regional scale. However this relationship is not found in all studies (Dauber et al., 2003; Krauss et al., 2004). These results seem dependent on the scale at which landscape heterogeneity is evaluated (Söderström et al., 2001). Some studies also showed an important effect of the species regional pool on diversity at the plot scale. Heterogeneity at the regional scale might therefore increase diversity at the plot scale by increasing the pool of species and therefore the possibility of recruitment from diaspores from the surroundings (Tilman, 1997). Habitat fragmentation is an important aspect of the landscape structure (Piessens et al., 2004). The effect of fragmentation can be split in tow process: the size of the fragment and the edge effect. The smaller the fragments of an habitat are, the lower is the number of species that can be found within this habitat (MacArthur and Wilson, 1967; Roschewitz et al., 2005). However, habitat fragmentation creates a lot of edges. Edges can enhance biodiversity by increasing the opportunities for communities including species originating from the two different adjacent habitats (Aude et al., 2003; Fédoroff et al., 2005). Connectivity between fragments also allows increasing specific richness (Hilty et al., 2006).

Plant diversity is favored by low management intensity, while medium management intensity hardly improves biodiversity compared to intensive management (Kleijn et al., 2009; Nemecek et al., 2011). A combination of plots managed at high and at very low intensity therefore appears more promising for maintaining biodiversity than management at medium intensity on the whole farmland area. Moreover, grasslands managed at different intensity shelter different plant communities with to some extent different species, and habitat heterogeneity is recognized as favorable for biodiversity at regional scale (e.g.

Benton et al., 2003). For instance, of the 189 plant species found by Marini et al. (2008) in 45 meadows, 77 were found exclusively under extensive management, 4 exclusively under medium and 9 exclusively under high management intensity. But species richness of grassland plants is also positively influenced by the surface area of the habitat (Krauss et al., 2004). The size and the location of the plots should therefore be considered to maximize habitat area and connectivity within and between farms (Knop et al., 2011). The Swiss agri-environment scheme requests that farmers allocate a minimum of 7 % of their agricultural area to ecological focus areas, in the mountains mostly extensive grasslands. Kampmann et al. (2012) showed that this scheme contributes to protection of plant diversity in mountain grasslands, despite the fact that the size of these species-rich plots is modest. The role of grassland areas within arable cropping systems for biodiversity conservation was recently discussed by Bretagnolle et al. (2011). These authors concluded that grasslands can play an important role for organisms of different trophic levels in landscapes dominated by arable crops. But they pointed out that to fulfill this role, grasslands should, at the landscape level, be managed according to the objective of biodiversity conservation.

The effects of the landscape can be as important as the effects of agricultural management or soil conditions (Gaujour, 2010, Lomba et al., 2011). From a functional point of view, landscape has a strong effect on the dispersion and propagation traits (seed traits), but for the other functional aspect (life strategies, leaf an root traits), only the effects of landscape on life strategies was studied (Gaujour, 2010, Lomba et al., 2011) and the processes behind these effects remain poorly known.

The second question concerns the characterization of biodiversity at the landscape level. This is crucial because some services or functions are linked to the diversity at the landscape level rather than at the plot level. Many pollinators need a diversity of habitats to feed through the seasons and/or to fulfill their life cycle. At the farm scale, some diversity of phenology can be useful to agricultural production. This indeed allows the farmers staggering the use of their grasslands, increasing the time of grazing and decreasing the workload during spring. As discussed above, such diversity can be amplified by a differentiated management of the grassland surfaces at the farm scale.

For specific richness can be decomposed into alpha, beta and gamma diversity. Alpha diversity is defined as the specific richness at the plot level. Gamma diversity is defined as the specific richness at the regional/landscape level. Beta diversity can defined as the differences between the gamma diversity and the average alpha diversity or as the gamma diversity divided by the alpha diversity (Whittaker, 1975). For functional traits, the decomposition is more difficult. Decomposition protocol is only proposed for the Rao indexes (Functional dispersion; Ricotta and Szeidl, 2009; de Bello et al., 2010a; de Bello et al., 2011). In grassland, this decomposition showed that most of the functional dispersion of the SLA is found at the plot Level (de Bello et al., 2009). Up-scaling to the regional scale did not increase the dispersion of the SLA. However this study was conducted only in one region. For aggregated trait, one approach is to produce maps of the studied functional trait (Lavorel et al., 2011). Such maps are used to evaluate ecosystem services. For example, it would be useful on aggregated trait to calculate the average aggregate trait of all the plots, the difference between the two extreme and the standard deviation.

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Annex II: A Grass Use Intensity index to be used across regions and grassland managements

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A. Abstract

The widely-used concept of intensity of grassland management can be defined according to fertilization level and defoliation frequency for mown meadows or to the stocking rate for pastures. Many questions arise for comparing situations over a large gradient of pedo-climatic conditions, or grazing, mowing and mixed grass utilizations. We therefore propose an index of intensity of grassland management combining grazing and mowing, and considering regional differences in biomass productivity. A model predicting the percentage of grass eaten by the animals as a function of the stocking rate was developed based on field measurements. The index can sum this percentage of grass defoliated by grazing to that defoliated by cutting, considering that one cut defoliates 100% of the vegetation. Regional differences in biomass productivity are taken into account by dividing the sum of defoliations by the biomass productivity of grassland at the regional level, which is estimated from remote sensing images. This index could be used to roughly estimate management intensity when field measurements are not available and a large range of situations have to be compared

Keywords: Mixed grassland, Number of cuts, Remote sensing, Stocking rate

B. Introduction

Intensity of grassland management is a key parameter for assessing the effects of agriculture on grassland biodiversity. Difficulties arise when it has to be quantified over a broad range of grassland types. First, comparing mown and grazed plots or estimating the intensity of grassland management when plots are alternatively mown and grazed require estimating the proportion of standing biomass removed by the grazing events (Lienin and Kleyer, 2012). However, this proportion is rarely known. Second, the effects of the number of defoliations on grassland vegetation depend on the length of vegetation period and of other abiotic factors influencing plant growth. Quantifying management intensity in different regions therefore requires accounting for differences in the potential of biomass production across regions. Herzog et al. (2006) proposed to normalize the mowing and grazing intensity by the maximum of these two intensities at the regional scale. This approach is very sensitive to the determination of these maximums and thus to the definition of regions and to sampling effort. Our aim is here to propose a methodology for calculating an index of intensity of grass use that could be used over a large gradient of pedo-climatic conditions and plot utilization. We use the term 'Grass Use Intensity index' (R*GU*) and not 'intensity of grassland management' because the level of fertilization is not aggregated to the frequency of defoliation in the proposed index.

C. Materials and Methods

The percentage of vegetal cover defoliated (*%Def*) at each mowing or grazing cycle is first estimated to calculate a defoliation index. It is considered that 100% of the vegetation is

defoliated at each mowing event. For grazing, *%Def* was estimated from the stocking rate based on sward height measurements in a field experiment comparing the effects of different grazing intensities in France, UK and Germany (Dumont et al. 2007, Isselstein et al. 2007). At the French site, the percentage of grazed patches was also recorded, which showed that the percentage of vegetation below 12 cm would be a good indicator of the percentage of grazed grass (data not shown). This percentage was thus used to determine the effect of the stocking rate (LU·days ha-1) on *%Def* by grazing in the three sites. The defoliation index was then obtained by summing the percentage of defoliation in successive plot utilizations along the grazing season. We propose to use the Normalized Differenced Vegetation Index (NDVI) from remote sensing images to estimate potential grass production (Ppot) at each site (Paruelo et al. 1997). NDVI values were obtained from MODIS satellites images (250 m·250 m pixel, one image every 16 days) and were filtered using the protocol of. Taugourdeau et al. (2010). A model to estimate grass production from the yearly dynamic of NDVI was constructed using production data from 217 grasslands in France and Switzerland. To facilitate the interpretation of the absolute values of the RGU, the index could be scaled with a reference yield and corresponding number of cuts for an intensive utilization system, for instance 5 cuts (Σ %Def = 500) for a production of 12 t DM ha-1 y-1. The RGU is thus calculated as:

$$RGU = \frac{\sum \%Def}{P_{pot}}\left(\frac{12}{500}100\right)$$

D. Results and Discussion

The relationship between the number of LU·days ha^{-1} during one grazing cycle and the percentage of vegetation below 12 cm was found to be: *%12cm* = 16.28*ln* (LU·days ha^{-1}) – 10.3 (R^2 = 0.70; Fig. 1) for the less productive site in France (F), and *%12cm* = 20.60*ln* (LU·days ha^{-1}) – 44.6 (R^2 = 0.69) for the more productive sites in Germany (G) and the UK. The difference between sites is probably due to the grazing behavior of cattle that were shown to increase their selectivity for short vegetative regrowths in the most productive grasslands (Dumont *et al.*, 2007). This could explain the lower percentage of plot cover that was considered as being grazed for a given stocking rate in G and in the UK compared with F.

Productivity of the 217 grasslands with yield data was best predicted from the dynamic of the NDVI of the pixels corresponding to the locations of the grasslands by the equation: P_{pot} = 11.9$NDVI_{feb}$ + 6.9$NDVI_{sep}$ – 14$NDVI_{nov}$ + 4.8 (R^2=0.38). Examples of R*GU* calculated for some scenarios using the above proposed equations are given in Table 1. For a grassland situated at a location with a P_{pot} of 7.2 t DM ha^{-1} y^{-1} (e.g. upland areas), 3 cuts per year correspond to a *RGU* of 100, while for the same number of cuts at a location with a P_{pot} of 12 t DM ha^{-1} y^{-1} (lowland) the R*GU* would be of 60. Grazing 770 LU·days (which corresponds to a grass consumption of 10 t DM) in 7 grazing cycles at the location with P_{pot} =12, yield a R*GU* of 73, although this corresponds to an intensive grazing system. The lower R*GU* calculated for intensive grazing than for intensive cutting is due to the fact that the percentage of cover defoliated by grazing animals never reaches 100% even in the most intensive systems.

This paper proposes a methodology for calculating an index that could quantify grass use intensity over a large gradient of pedo-climatic conditions and for different types of utilization. Combining information from remote sensing images to estimate the potential grass production across regions with an empirical model to estimate the proportion of plot cover defoliated during grazing events allows such comparisons. The relationship between stocking rate and percentage of defoliated cover as well as the estimation of the potential

grass production from NDVI are based on a small number of data, and will have to be validated before the proposed *RGU* can be widely used.

Table 1: Calculated Index of Grass Use Intensity (*RGU*) for locations with different potential of production (P_{pot}) under different agricultural management.

P_{pot} (t DM ha^{-1})	Cuts	LU·days	Graz. cycles	Σ%Def	RGU
12.0	5			500	100
12.0	3			300	60
12.0		770	7	366	73
12.0	3	330	3	457	91
7.2	3			300	100
7.2		440	4	265	88

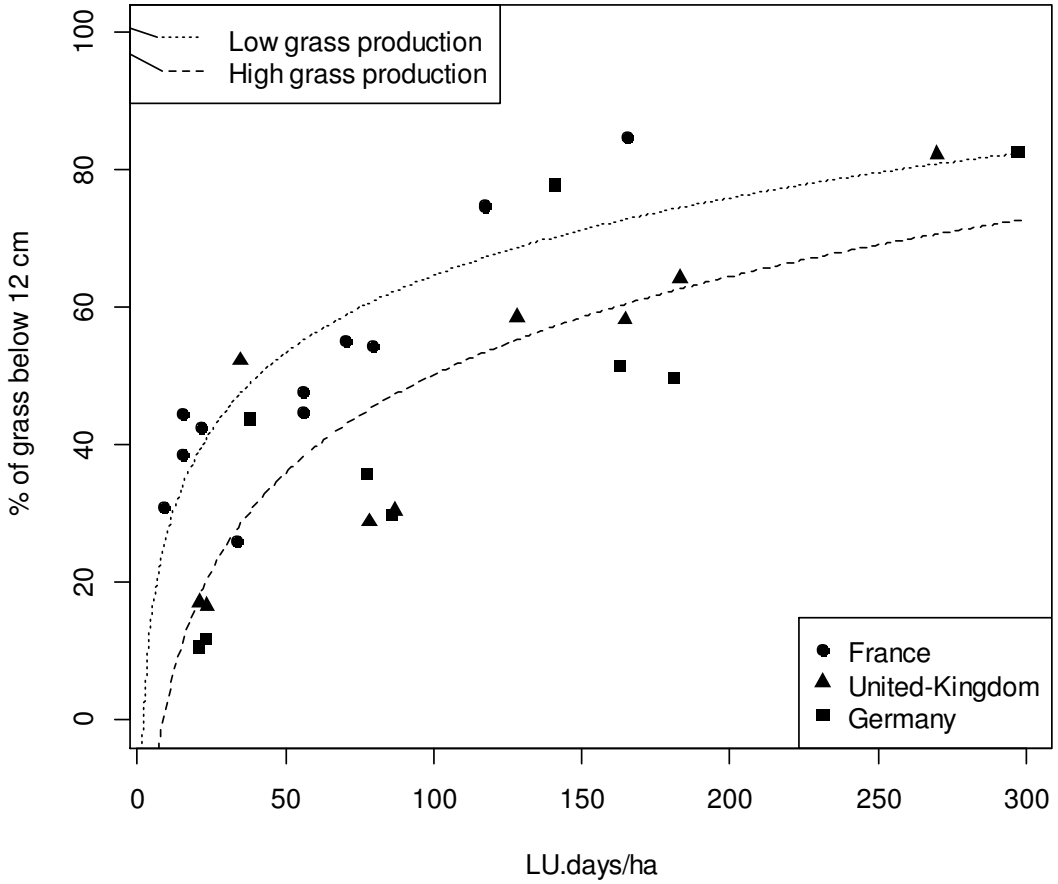

Figure1: Relationships between stocking rate and percentage of cover below 12 cm for each grazing cycle based on three sites in F: France, U: United-Kingdom and G: Germany.

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Effects of management and climate on the plant functional diversity related to ecosystem services of permanent grasslands in Europe

Abstract
Permanent grasslands can provide a large diversity of ecosystem services. They are found in contrasted conditions in terms of management and climate in Europe. These conditions induce a wide variety of grassland types that differ in their vegetation and are expected to differ with respect to the provision of ecosystem services. Because ecosystem functioning in grassland has been shown to be linked to plant functional diversity criteria, we put forward that these functional diversity criteria can be used to evaluate the provision of ecosystem services. These criteria are influenced by the management and the climate. The overall objective of this PhD program is to assess the effects of management and climate on the plant functional diversity related to ecosystem services of permanent grasslands. The first step of the PhD program was to select 29 functional diversity criteria related to 8 ecosystem services (quantity of forage, forage quality, stability of the forage production, biodiversity conservation, pollination carbon sequestration, dryness resistance and nitrogen fertility). This selection was made using experts' interview and literature survey. The second step was to evaluate inaccuracies in the calculation of the 29 functional diversity criteria from functional trait databases and different sets of botanical surveys. The third step was to estimate the functional diversity criteria from management and climate data using “Random Forests” methodology. The results show that the climatic variables had generally more effect on the functional diversity than the management ones. Furthermore, for some climatic conditions, the management does not affect some functional diversity criteria. Among the 29 functional diversity criteria, only 8 have more than 40 % of their variance explained by the management and climate variables.
Keywords Functional diversity, climate, management, ecosystem services, permanent grasslands

Effets de la gestion et du climat sur la diversité fonctionnelle végétale reliée aux services écosystémiques des prairies permanentes en Europe

Résumé
Les prairies permanentes peuvent fournir une grande diversité de services écosystémiques. Elles se trouvent dans des conditions contrastées en termes de gestion et de climat en Europe. Cette diversité de conditions induit une grande variété de types de prairies différant par leur végétation et leur fourniture en services écosystémiques. Certains travaux suggèrent que le fonctionnement des écosystèmes prairiaux peut être directement relié à des critères de diversité fonctionnelle végétale. Ces critères, qui pourraient être utilisés pour évaluer les services écosystémiques, sont influencés par la gestion et le climat. L'objectif de ce doctorat est d'évaluer les effets de la gestion et du climat sur la diversité fonctionnelle végétale liée à des services écosystémiques des prairies permanentes. La première étape a consisté à sélectionner 29 critères de diversité fonctionnelles liés à 8 services écosystémiques (quantité de fourrage, qualité du fourrage, stabilité de la production de fourrage, conservation de la biodiversité, pollinisation, la séquestration du carbone, résistance à la sécheresse et fertilité azotée). Cette sélection a été faite à partir d’interviews d’experts et d’une revue de la littérature. La deuxième étape a consisté à évaluer différentes inexactitudes dans le calcul des 29 critères de diversité fonctionnelle à partir de bases de traits fonctionnels et différents jeux de relevés botaniques. La troisième étape a consisté à évaluer les critères de diversité fonctionnelle à partir des données de gestion et de climat, en utilisant notamment la technique mathématique des forêts de Breiman. Les résultats montrent que les variables climatiques ont généralement plus d'effet sur la diversité fonctionnelle que celles de gestion. En outre, pour certaines conditions climatiques, la gestion n'affecte pas certains critères de diversité fonctionnelle. Parmi les 29 critères de diversité fonctionnelle, seulement 8 critères avaient plus de 40% de leur variance expliquée par la gestion et le climat..
Mots clés: diversité fonctionnelle, climat, gestion agricole, services écosystémiques prairies permanentes